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Application for Patent

אני, (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)
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רחובות

בעל אמצאה מכח העברה ששמה הוא Assignment
Owner, by virtue of ... of an invention the title of which is

(בעברית) (Hebrew) מודולטורים של קולטנים ממשפחת-העל TNF/NGF, הכנתם והשימוש בהם

(באנגלית) (English) MODULATORS OF TNF/NGF SUPERFAMILY RECEPTORS, THEIR PREPARATION AND USE

hereby apply for a patent to be granted to me in respect thereof.
מבקש בזאת כי ינתן לי עליה פטנט

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היום 19 בחודש פברואר שנת 1995
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112692

MODULATORS OF TNF/NGF SUPERFAMILY RECEPTORS, THEIR PREPARATION AND USE

מודולטורים של קולטנים ממשפחת-העל TNF/NGF הכנתם והשימוש בהם

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Field of the Invention

The present invention is generally in the field of receptors belonging to the TNF/NGF superfamily of receptors and the control of their biological functions. The TNF/NGF superfamily of receptors includes receptors such as the p55 and p75 tumor necrosis factor receptors (TNF-Rs) and the FAS ligand receptor (also called FAS/APO1 or FAS-R and hereinafter will be called FAS-R) and others. More specifically, the present invention concerns novel proteins which bind to the intracellular domains (IC) of the p55 and p75 TNF-Rs and the Fas-R, (these intracellular domains designated p55IC, p75IC and Fas-IC, respectively) and which novel proteins are capable of modulating the function of the p55 and p75 TNF-Rs and the Fas-R. Further, one of these novel intracellular domain-binding proteins, herein designated HF1 or MORT-1, which binds specifically to FAS-IC, is also capable of self-association and can activate cell cytotoxicity on its own. Accordingly, the present invention also concerns proteins capable of binding to HF1 which are capable of modulating the function of HF1. The present invention also concerns the preparation and uses of these novel p55 and p75 TNF-R-binding proteins, and Fas-R binding proteins, referred to herein as p55IC-, p75IC- and Fas-IC- binding proteins.

Background of the Invention and Prior Art

The present application is an application for a Patent of Addition to the co-pending Israel Patent Application No. 109632 and is related also to co-pending Israel applications 111125 and 112002, in which there are disclosed, among other aspects, new proteins which are capable of binding to either the intracellular domain of the p75 TNF-R or the intracellular domain of the p55 TNF-R (p75 IC- and p55-IC-binding proteins respectively); as well as the fact that the p55 IC and FAS-IC are capable of self-association and contain within their IC domain a region called the 'death domain' (DD) which is primarily responsible for this self-association. Thus, both the p55IC and FAS-IC as well as the p55IC and FAS-IC death domains (p55DD and FAS-DD, respectively) also represent p55IC- and FAS-IC- binding proteins capable of mediating the function of these receptors.

The present invention concerns proteins which can bind to the intracellular domains of receptors belonging to the TNF/NGF family in general, and proteins capable of binding to the p55IC, p75IC and FAS-IC in particular. These IC-binding proteins include new proteins as well as portions, e.g. the p55IC, p55DD, FAS-IC and FAS-DD, of intracellular domains of the various TNF/NGF receptors that are capable of binding TNF/NGF receptor intracellular domains.

Tumor Necrosis Factor (TNF-α) and Lymphotoxin (TNF-β) (hereinafter, TNF, refers to both TNF-α and TNF-β) are multifunctional pro-inflammatory cytokines formed mainly by mononuclear phagocytes, which have many effects on cells (Wallach, D. (1986) in : Interferon 7 (Ion Gresser, ed.), pp. 83-122, Academic Press, London; and Beutler and Cerami (1987)). Both TNF-α and TNF-β initiate their effects by binding to specific cell surface receptors. Some of the effects are likely to be beneficial to the organism : they may destroy, for example tumor cells or virus infected cells and augment antibacterial activities of granulocytes. In this way, TNF contributes to the defense of the organism against tumors and infectious agents and contributes to the recovery from injury. Thus, TNF can be used as an anti-tumor agent in which application it binds to its receptors on the surface of tumor cells and thereby initiates the events leading to the death of the tumor cells. TNF can also be used as an anti-infectious agent.

However, both TNF-α and TNF-β also have deleterious effects. There is evidence that over-production of TNF-α can play a major pathogenic role in several diseases. Thus, effects of TNF-α, primarily on the vasculature, are now known to be a major cause for symptoms of septic shock (Tracey et al., 1986). In some diseases, TNF may cause excessive loss of weight (cachexia) by suppressing activities of adipocytes and by causing anorexia, and TNF-α was thus called cachetin. It was also described as a mediator of the damage to tissues in rheumatic diseases (Beutler and Cerami, 1987) and as a major mediator of the damage observed in graft-versus-host reactions (Piquet et al., 1987). In addition, TNF is known to be involved in the process of inflammation and in many other diseases.

Two distinct, independently expressed, receptors, the p55 and p75 TNF-Rs, which bind both TNF-α and TNF-β specifically, initiate and/or mediate the above noted biological effects of TNF. These two receptors have structurally dissimilar intracellular domains suggesting that they signal differently (See Hohmann et al., 1989; Engelmann et al., 1990; Brockhaus et al., 1990; Leotscher et al., 1990; Schall et al., 1990; Nophar et al., 1990; Smith et al., 1990; and Heller et al., 1990). However, the cellular mechanisms, for example, the various proteins and possibly other factors, which are involved in the intracellular signaling of the p55 an p75 TNF-Rs have yet to be elucidated (In IL 109632 and as set forth also herein below, there is described for the first time, new proteins capable of binding to the p75IC and p55 IC). It is this intracellular signaling, which occurs usually after the binding of the ligand, i.e. TNF (α or β), to the receptor, that is responsible for the commencement of the cascade of reactions that ultimately result in the observed response of the cell to TNF.

As regards the above mentioned cytocidal effect of TNF, in most cells studied so far, this effect is triggered mainly by the p55 TNF-R. Antibodies against the extracellular domain (ligand binding domain) of the p55 TNF-R can themselves trigger the cytocidal effect (see EP 412486) which correlates with the effectivity of receptor cross-linking by the antibodies, believed to be the first step in the generation of the intracellular signaling process. Further, mutational studies (Brakebusch et al., 1992; Tartaglia et al., 1993) have shown that the biological function of the p55 TNF-R depends on the integrity of its intracellular domain, and accordingly it has been suggested that the initiation of intracellular signaling leading to the cytocidal effect of TNF occurs as a consequence of the association of two or more intracellular domains of the p55 TNF-R. Moreover, TNF (α and β) occurs as a homotrimer and as such has been suggested to induce intracellular signaling via the p55 TNF-R by way of its ability to bind to and to cross-link the receptor molecules, i.e. cause receptor aggregation. In IL 109632 and IL 111125 and also herein below there is described how the p55IC and p55DD can self-associate and induce, in a ligand-independent fashion, TNF-associated effects in cells.

Another member of the TNF/NGF superfamily of receptors is the FAS receptor (FAS-R) which has also been called the Fas antigen, a cell-surface protein expressed in various tissues and sharing homology with a number of cell-surface receptors including TNF-R and NGF-R. The FAS-R mediates cell death in the form of apoptosis (Itoh et al., 1991), and appears to serve as a negative selector of autoreactive T cells, i.e. during maturation of T cells, FAS-R mediates the apoptopic death of T cells recognizing self-antigens. It has also been found that mutations in the FAS-R gene (*lpr*) cause a lymphoproliferation disorder in mice that resembles the human autoimmune disease systemic lupus erythematosus (SLE) (Watanabe-Fukunaga et al., 1992). The ligand for the FAS-R appears to be a cell-surface associated molecule carried by, amongst others, killer T cells (or cytotoxic T lymphocytes - CTLs), and hence when such CTLs contact cells carrying FAS-R, they are capable of inducing apoptopic cell death of the FAS-R-carrying cells. Further, a monoclonal antibody has been prepared that is specific for FAS-R, this monoclonal antibody being capable of inducing apoptopic cell death in cells carrying FAS-R, including mouse cells transformed by cDNA encoding human FAS-R (Itoh et al., 1991).

It has also been found that various other normal cells, besides T lymphocytes, express the FAS-R on their surface and can be killed by the triggering of this receptor. Uncontrolled induction of such a killing process is suspected to contribute to tissue damage in certain diseases, for example, the destruction of liver cells in acute hepatitis. Accordingly, finding ways to restrain the cytotoxic activity of FAS-R may have therapeutic potential.

Conversely, since it has also been found that certain malignant cells and HIV-infected cells carry the FAS-R on their surface, antibodies against FAS-R, or the FAS-R ligand, may be used to trigger the FAS-R mediated cytotoxic effects in these and thereby provide a means for combating such malignant cells or HIV-infected cells (see Itoh et al., 1991). Finding yet other ways for enhancing the cytotoxic activity of FAS-R may therefore also have therapeutic potential.

It has been a long felt need to provide a way for modulating the cellular response to TNF (α or β) and FAS-R ligand, for example, in pathological situations as mentioned

above, where TNF or FAS-R ligand is over-expressed it is desirable to inhibit the TNF- or FAS-R ligand- induced cytocidal effects, while in other situations, e.g. wound healing applications, it is desirable to enhance the TNF effect, or in the case of FAS-R, in tumor cells or HIV-infected cells it is desirable to enhance the FAS-R mediated effect.

A number of approaches have been made by the present inventors (see for example, European Application Nos. EP 186833, EP 308378, EP 398327 and EP 412486) to regulate the deleterious effects of TNF by inhibiting the binding of TNF to its receptors using anti-TNF antibodies or by using soluble TNF receptors (being essentially the soluble extracellular domains of the receptors) to compete with the binding of TNF to the cell surface-bound TNF-Rs. Further, on the basis that TNF-binding to its receptors is required for the TNF-induced cellular effects, approaches by the present inventors (see for example IL 101769 and its corresponding EPO 568925) have been made to modulate the TNF effect by modulating the activity of the TNF-Rs. Briefly, EPO 568925 (IL 101769) relates to a method of modulating signal transduction and/or cleavage in TNF-Rs whereby peptides or other molecules may interact either with the receptor itself or with effector proteins interacting with the receptor, thus modulating the normal functioning of the TNF-Rs. In EPO 568925 there is described the construction and characterization of various mutant p55 TNF-Rs, having mutations in the extracellular, transmembranal, and intracellular domains of the p55 TNF-R. In this way regions within the above domains of the p55 TNF-R were identified as being essential to the functioning of the receptor, i.e. the binding of the ligand (TNF) and the subsequent signal transduction and intracellular signaling which ultimately results in the observed TNF-effect on the cells. Further, there is also described a number of approaches to isolate and identify proteins, peptides or other factors which are capable of binding to the various regions in the above domains of the TNF-R, which proteins, peptides and other factors may be involved in regulating or modulating the activity of the TNF-R. A number of approaches for isolating and cloning the DNA sequences encoding such proteins and peptides; for constructing expression vectors for the production of these proteins and peptides; and for the preparation of antibodies or fragments thereof which interact with the TNF-R or with the above proteins

and peptides that bind various regions of the TNF-R, are also set forth in EPO 568925. However, no description is made in EPO 568925 of the actual proteins and peptides which bind to the intracellular domains of the TNF-Rs (e.g. p55 TNF-R), nor is any description made of the yeast two-hybrid approach to isolate and identify such proteins or peptides which bind to the intracellular domains of TNF-Rs. Similarly, heretofore there has been no disclosure of proteins or peptides capable of binding the intracellular domain of FAS-R.

Thus, when it is desired to inhibit the effect of TNF, or the FAS-R ligand, it would be desirable to decrease the amount or the activity of TNF-Rs or FAS-R at the cell surface, while an increase in the amount or the activity of TNF-Rs or FAS-R would be desired when an enhanced TNF or FAS-R ligand effect is sought. To this end the promoters of both the p55 TNF-R and the p75 TNF-R have been sequenced, analyzed and a number of key sequence motifs have been found that are specific to various transcription regulating factors, and as such the expression of these TNF-Rs can be controlled at their promoter level, i.e. inhibition of transcription from the promoters for a decrease in the number of receptors, and an enhancement of transcription from the promoters for an increase in the number of receptors (see IL 104355 and IL 109633). Corresponding studies concerning the control of FAS-R at the level of the promoter of the FAS-R gene have yet to be reported.

Further, it should also be mentioned that, while it is known that the tumor necrosis factor (TNF) receptors, and the structurally-related receptor FAS-R, trigger in cells, upon stimulation by leukocyte-produced ligands, destructive activities that lead to their own demise, the mechanisms of this triggering are still little understood. Mutational studies indicate that in FAS-R and the p55 TNF receptor (p55-R) signaling for cytotoxicity involve distinct regions within their intracellular domains (Brakebusch et al., 1992; Tartaglia et al., 1993; Itoh and Nagata, 1993). These regions (the 'death domains') have sequence similarity. The 'death domains' of both FAS-R and p55-R tend to self-associate. Their self-association apparently promotes that receptor aggregation which is necessary for initiation of signaling (see IL 109632, IL 111125 and IL 112002, as well as Song et al., 1994; Wallach et al., 1994; Boldin et al., 1995) and at high levels of receptor expression

can result in triggering of ligand-independent signaling (IL 109632, IL 111125 and Boldin et al., 1995).

Thus, prior to IL 109632 and the present invention, there have not been provided proteins which may regulate the effect of ligands belonging to the TNF/NGF superfamily, such as the TNF or FAS-R ligand effect on cells, by mediation of the intracellular signaling process, which signaling is probably governed to a large extent by the intracellular domains (ICs) of the receptors belonging to the TNF/NGF superfamily of receptors, such as those of the TNF-Rs, i.e. the p55 and p75 TNF-R intracellular domains (p55IC and p75IC, respectively), as well as the FAS-IC.

Accordingly, it is one aim of the invention to provide proteins which are capable of binding to the intracellular domains of the TNF-Rs and FAS-R, which proteins are presently believed to be involved in the intracellular signaling process initiated by the binding of TNF to its receptors, or the binding of FAS ligand to its receptor.

Another aim of the invention is to provide antagonists (e.g. antibodies) to these intracellular domain-binding proteins (IC-binding proteins) which may be used to inhibit the signaling process, when desired, when such IC-binding proteins are positive signal effectors (i.e. induce signaling), or to enhance the signaling process, when desired, when such IC-binding proteins are negative signal effectors (i.e. inhibit signaling).

Yet another aim of the invention is to use such IC-binding proteins to isolate and characterize additional proteins or factors, which may, for example, be involved further downstream in the signaling process, and/or to isolate and identify other receptors further upstream in the signaling process to which these IC-binding proteins bind (e.g. other TNF-Rs or related receptors), and hence, in whose function the IC-binding proteins are also involved.

Moreover, it is an aim of the present invention to use the above-mentioned IC-binding proteins as antigens for the preparation of polyclonal and/or monoclonal antibodies thereto. The antibodies, in turn, may be used for the purification of the new IC-binding proteins from different sources, such as cell extracts or transformed cell lines.

Furthermore, these antibodies may be used for diagnostic purposes, e.g. for identifying disorders related to abnormal functioning of cellular effects mediated by receptors belonging to the TNF/NGF receptor superfamily.

A further aim of the invention is to provide pharmaceutical compositions comprising the above IC-binding proteins, and pharmaceutical compositions comprising the IC-binding protein antagonists, for the treatment or prophylaxis of TNF-induced or FAS ligand-induced conditions, for example, such compositions can be used to enhance the TNF or FAS ligand effect or to inhibit the TNF or FAS ligand effect depending on the above noted nature of the IC-binding protein or antagonist thereof contained in the composition.

Summary of the Invention

In accordance with the present invention, we have found novel proteins which are capable of binding to either the intracellular domain of the p55 TNF-R (the p55IC-binding proteins), of the p75 TNF-R (the p75IC-binding proteins), and of the FAS-R (the FAS-IC-binding proteins). These p55IC-, p75IC- and FAS-IC- binding proteins may act as mediators or modulators of the TNF or FAS-R ligand effect on cells by way of mediating or modulating the intracellular signaling process which usually occurs following the binding of TNF to the p55 and/or p75 TNF-R, or the binding of the FAS-R ligand at the cell surface. Further, it has been surprisingly and unexpectedly found that the p55IC and FAS-IC are capable of self association and that fragments of the p55IC and FAS-IC are similarly capable of binding to the p55 IC, particularly the so-called 'death domains (DD) within the ICs of these receptors, i.e. the p55DD and FAS-DD. Thus, p55 IC and FAS-IC and their fragments also represent proteins capable of binding to the p55IC and FAS-IC and hence may be modulators of the TNF or FAS-R ligand effect on cells.

Moreover, it has now also been found that one of the intracellular domain-binding proteins of the invention, namely, HF1 (also called MORT-1 for 'Mediator of Receptor Toxicity'), which binds specifically to the Fas-IC, has, in addition to other characteristics (see Example 3), a region homologous to the 'death domain' (DD) regions of the p55-

TNF-R and FAS-R (p55-DD and FAS-DD), and thereby is also capable of self-association. HF1 is also capable of activating cell cytotoxicity on its own, an activity possibly related to its self-association capability. Accordingly, it is likely that HF1 also binds to other proteins involved in the intracellular signaling process. These HF1-binding proteins may therefore also act as indirect mediators or modulators of the FAS-R ligand effect on cells by way of mediating or modulating the activity of HF1; or these HF1-binding proteins may act directly as mediators or modulators of the HF1-associated intracellular signaling process by way of mediating or modulating the activity of HF1, which, as noted above, has an apparently independent ability to activate cell cytotoxicity. These HF1-binding proteins may also be used in any of the standard screening procedures to isolate, identify and characterize additional proteins, peptides, factors, antibodies, etc. which may be involved in the HF1-associated or FAS-R-associated signaling process or may be elements of other intracellular signaling processes.

Accordingly, the present invention provides a DNA sequence encoding a protein capable of binding to one or more of the intracellular domains of one or more receptors belonging to the tumor necrosis factor/nerve growth factor (TNF/NGF) superfamily of receptors.

In particular, the present invention provides a DNA sequence selected from the group consisting of :

(a) a cDNA sequence derived from the coding region of a native TNF-R intracellular domain-binding protein;

(b) DNA sequences capable of hybridization to a DNA of (a) under moderately stringent conditions and which encode a biologically active TNF-R intracellular domain-binding protein; and

(c) DNA sequences which are degenerate as a result of the genetic code to the DNA sequences defined in (a) and (b) and which encode a biologically active TNF-R intracellular domain-binding protein.

The present invention also provides a DNA sequence selected from the group consisting of :

(a) a cDNA sequence derived from the coding region of a native FAS-R intracellular domain-binding protein;

(b) DNA sequences capable of hybridization to a cDNA of (a) under moderately stringent conditions and which encode a biologically active FAS-R intracellular domain-binding protein; and

(c) DNA sequences which are degenerate as a result of the genetic code to the DNA sequences defined in (a) and (b) and which encode a biologically active FAS-R intracellular domain-binding protein.

In embodiments of the present invention the DNA sequences encode p55 TNF-R, p75 TNF-R and FAS-R intracellular domain-binding proteins, such as those encoding the herein designated proteins 55.1, 55.3, 55.11, 75.3, 75.16, HF1 (MORT-1), F2, F9 and DD11.

The present invention also provides a protein or analogs or derivatives thereof encoded by any of the above sequences of the invention, said proteins, analogs and derivatives being capable of binding to one or more of the intracellular domains of one or more TNF-Rs or FAS-R. Embodiments of this aspect of the invention include the herein designated proteins 55.1, 55.3, 55.11, 75.3, 75.16, HF1 (MORT-1), F2, F9 and DD11, their analogs and their derivatives.

Also provided by the present invention are vectors encoding the above proteins of the invention, which contain the above DNA sequences of the invention, these vectors being capable of being expressed in suitable eukaryotic or prokaryotic host cells; transformed eukaryotic or prokaryotic host cells containing such vectors; and a method for producing the proteins, analogs or derivatives of the invention by growing such transformed host cells under conditions suitable for the expression of said protein, analogs or derivatives, effecting post-translational modifications of said protein as necessary for obtention of said protein and extracting said expressed protein, analogs or derivatives from the culture medium of said transformed cells or from cell extracts of said transformed cells.

In another aspect, the present invention also provides antibodies or active derivatives or fragments thereof specific to the proteins, analogs and derivatives thereof, of the invention.

By yet another aspect of the invention, there are provided various uses of the above DNA sequences or the proteins which they encode, according to the invention, which uses include amongst others :

(i) a method for the modulation of the TNF or FAS-R ligand effect on cells carrying a TNF-R or a FAS-R, comprising treating said cells with one or more proteins, analogs or derivatives selected from the group consisting of the proteins, analogs and derivatives, according to the invention, and a protein being the p55IC, p55DD, FAS-IC or FAS-DD, analogs or derivatives thereof, all of said proteins being capable of binding to the intracellular domain and modulating the activity of said TNF-R or FAS-R, wherein said treating of the cells comprises introducing into said cells said one or more proteins, analogs or derivatives in a form suitable for intracellular administration or introducing into said cells, in the form of a suitable expression vector, the DNA sequence encoding said one or more proteins, analogs or derivatives ;

(ii) a method for modulating the TNF or FAS-R ligand effect on cells carrying a TNF-R or a FAS-R comprising treating said cells with antibodies or active derivatives or fragments thereof according to the invention;

(iii) a method for modulating the TNF or FAS-R ligand effect on cells carrying a TNF-R or FAS-R comprising treating said cells with an oligonucleotide sequence encoding an antisense sequence of at least part of the sequence according to the invention, or encoding an antisense sequence of the p55IC, p55DD, FAS-IC, or FAS-DD sequence, said oligonucleotide sequence being capable of blocking the expression of at least one of the TNF-R or FAS-R intracellular domain binding proteins;

(iv) a method for modulating the TNF or FAS-R ligand effect on cells carrying a TNF-R or FAS-R comprising :

(a) constructing a recombinant animal virus vector carrying a sequence encoding a viral surface protein that is capable of binding to a specific cell surface receptor and a sequence selected from an oligonucleotide sequence encoding an antisense sequence of at least part of the sequence according to the invention and an oligonucleotide sequence encoding an antisense sequence of the p55IC, p55DD, FAS-IC, or FAS-DD sequence, said oligonucleotide sequence being capable of blocking the expression of at least one of the TNF-R or FAS-R intracellular domain binding proteins when introduced into said cells by said virus ; and

(b) infecting said cells with said vector of (a).

(v) a method for modulating the TNF or FAS-R ligand effect on cells carrying a TNF-R or a FAS-R, comprising treating said cells with a suitable vector encoding a ribozyme having a sequence specific to a sequence selected from an mRNA sequence encoding a protein, analog or derivative of the invention and an mRNA sequence encoding the p55IC, p55DD, FAS-IC or FAS-DD, said ribozyme sequence capable of interacting with said mRNA sequence and capable of cleaving said mRNA sequence resulting in the inhibition of the expression of the protein, analog or derivative of the invention or of the expression of the p55IC, p55DD, FAS-IC or FAS-DD.

(vi) a method for treating tumor cells or HIV-infected cells, or other diseased cells, comprising:

(a) constructing a recombinant animal virus vector carrying a sequence encoding a viral surface protein that is capable of binding to a tumor cell surface receptor or HIV-infected cell surface receptor or is capable of binding to another cell surface receptor of other diseased cells and a sequence selected from a sequence according to the invention encoding a protein, analog or derivative of the invention and a sequence encoding the p55IC, p55DD, FAS-IC, FAS-DD, or a biologically active analog or derivative thereof, said protein, analog or derivative of the invention, p55IC, p55DD, FAS-IC, FAS-DD, analog or derivative, when

expressed in said tumor cell or HIV-infected cell, or other diseased cell being capable of killing said cell; and

(b) infecting said tumor cells or HIV-infected cells or other infected cells with said vector of (a).

(vii) a method for isolating and identifying proteins, factors or receptors capable of binding to the intracellular domain binding proteins according to the invention, comprising applying the procedure of affinity chromatography in which said protein according to the invention is attached to the affinity chromatography matrix, said attached protein is brought into contact with a cell extract and proteins, factors or receptors from cell extract which bound to said attached protein are then eluted, isolated analyzed;

(viii) a method for isolating and identifying proteins, capable of binding to the intracellular domain binding proteins according to the invention, comprising applying the yeast two-hybrid procedure in which a sequence encoding said intracellular domain binding protein is carried by one hybrid vector and a sequence from a cDNA or genomic DNA library is carried by the second hybrid vector, the vectors then being used to transform yeast host cells and the positive transformed cells being isolated, followed by extraction of the said second hybrid vector to obtain a sequence encoding a protein which binds to said intracellular domain binding protein; and

(ix) a method for isolating and identifying a protein capable of binding to the intracellular domains of TNF-Rs or FAS-R comprising applying the procedure of non-stringent southern hybridization followed by PCR cloning, in which a sequence or parts thereof according to the invention is used as a probe to bind sequences from a cDNA or genomic DNA library, having at least partial homology thereto, said bound sequences then amplified and cloned by the PCR procedure to yield clones encoding proteins having at least partial homology to said sequences according to the invention.

The present invention also provides a pharmaceutical composition for the modulation of the TNF- or FAS ligand- effect on cells comprising, as active ingredient, any one of the following : (i) a protein according to the invention, or the protein p55IC, p55DD, FAS-IC or FAS-DD, its biologically active fragments, analogs, derivatives or mixtures thereof; (ii) a recombinant animal virus vector encoding a viral surface protein capable of binding to a TNF-R or FAS-R - carrying cell - or tumor cell-specific receptor and a sequence encoding a protein, analog or derivative of the invention or encoding the p55IC, p55DD, FAS-IC or FAS-DD; (iii) a recombinant animal virus vector encoding a viral surface protein as in (ii) above and an oligonucleotide sequence encoding an antisense sequence of the p55IC, p55DD, FAS-IC or FAS-DD sequence; and (iv) a vector encoding a ribozyme of sequence capable of interacting with a mRNA sequence encoding a protein, analog or derivative of the invention or a mRNA sequence encoding the p55IC, p55DD, FAS-IC or FAS-DD.

Yet another aspect of the invention concerns, specifically, one of the above noted FAS-IC binding proteins of the invention, namely, the protein HF1 (MORT-1). By this aspect of the invention, as mentioned above, there is provided :

(i) a DNA sequence encoding the HF1 protein or analogs thereof, which are capable of binding to the intracellular domain of FAS-R; this DNA sequence being further characterized by being selected from the group of DNA sequences consisting of : a cDNA sequence derived from the coding region of a native HF1 protein; DNA sequences capable of hybridization to the above cDNA sequence under moderately stringent conditions and which encode biologically active HF1 proteins; and DNA sequences which are degenerate as a result of the genetic code to the above defined cDNA and DNA sequences and which encode a biologically active HF1 protein;

(ii) an HF1 protein, analogs or derivatives thereof, encoded by any of the above DNA sequences, which are capable of binding to FAS-R;

(iii) vectors encoding the above HF1 proteins which contain the above DNA sequences, these vectors being capable of being expressed in suitable eukaryotic or prokaryotic host cells;

(iv) transformed eukaryotic or prokaryotic host cells containing such vectors;

(v) a method for producing the HF1 proteins, analogs or derivatives, by growing the above transformed host cells under conditions suitable for the expression of the HF1 proteins, analogs or derivatives, effecting host translational modifications of said HF1 protein as necessary for obtention of said protein and extracting said expressed HF1 protein, analogs or derivatives from the culture medium of said transformed cells or from extracts of said transformed cells; and

(vi) antibodies or active derivatives or fragments thereof, specific to the HF1 proteins, analogs and derivatives thereof.

This aspect of the invention also includes various uses of the above DNA sequences encoding HF1 or the HF1 proteins encoded thereby, which uses include, amongst others, the hereinabove mentioned uses (i)-(ix), in which it is to be understood that these uses specifically concern the utilization of HF1, its analogs, or its derivatives for the modulation of FAS-R ligand effect (uses (i)-(v)), or for treating tumor cells, HIV-infected or other diseased cells (use (vi)), or for a method for isolating and identifying proteins, factors or receptors capable of binding to HF1 (uses (vii) and (viii)), or for a method for isolating and identifying a protein capable of binding to the FAS-R intracellular domain or a protein capable of binding to HF1 (use (ix)). It should be mentioned that HF1 has a distinct region which binds to the FAS-IC and another distinct region which is involved in self-association of HF1, and accordingly, these distinct regions or parts thereof may be used independently to identify other proteins, receptors, etc. which are capable of binding to HF1 or to FAS-R and which may be involved in the HF1- or FAS-R- related intracellular signaling processes. Further, HF1 may have other activities associated with either of the above distinct regions or other regions of HF1 or combinations thereof, for example, enzymatic activity, which may be related to the cell cytotoxic effects of HF1 on its own. Thus, HF1 may also be used to specifically identify other proteins, peptides, etc. which may be involved in such additional activities associated with HF1.

This aspect of the invention also includes a pharmaceutical composition for the modulation of the FAS ligand effect on cells comprising, as active ingredient, any one of the following :

(i) the HF1 protein, its biologically active fragments, analogs, derivatives or mixtures thereof;

(ii) a recombinant animal virus vector encoding a viral surface protein capable of binding to a FAS-R-carrying cell or tumor cell-specific receptor, and a sequence encoding the HF1 protein, analog, or derivative thereof; and

(iii) a vector encoding a ribozyme of sequence capable of interacting with a mRNA sequence encoding the HF1 protein, analog or derivative thereof.

In addition, this aspect of the invention also includes proteins which are capable of binding specifically to HF1. As set forth in Example 3 below, HF1 is capable of self-association and of activating cell cytotoxicity on its own. Thus, HF1 probably also binds to other proteins (besides FAS-R) which are involved in the intracellular signaling process. These HF1 binding proteins may act either as modulators of the HF1-associated intracellular signaling process or as modulators of the FAS-R ligand effect by modulating the activity of HF1.

Accordingly, the present invention also provides a DNA sequence encoding a protein capable of binding to HF1. In particular, the above HF1-binding protein sequence is selected from the group consisting of a cDNA sequence derived from a coding region of a native HF1-binding protein sequence; DNA sequences capable of hybridizing to the above cDNA sequence under moderately stringent conditions and which encode a biologically active HF1-binding protein; and DNA sequences which are degenerate as a result of the genetic code to the above cDNA and DNA sequences and which encode a biologically active HF1-binding protein.

Further, there is also provided an HF1-binding protein, analogs, or derivatives thereof encoded by any of the above sequences, said HF1-binding proteins, analogs and derivatives being capable of binding to HF1.

Moreover, there is also provided : vectors encoding the above HF1-binding proteins, which contain the HF1-binding protein encoding sequences, these vectors being capable of being expressed in suitable eukaryotic or prokaryotic host cells; transformed eukaryotic or prokaryotic host cells containing such vectors; and a method for producing the HF1-binding proteins, analogs or derivatives thereof, by growing such transformed host cells under conditions suitable for the expression of the HF1-binding protein, analogs or derivatives thereof, effecting post-translational modifications of the HF1-binding protein as necessary for obtention of said protein and extracting said expressed HF1-binding protein, analogs or derivatives from the culture medium of said transformed cells or from cell extracts of said transformed cells; and antibodies or active derivatives or fragments thereof specific to the HF1-binding proteins, analogs or derivatives thereof.

Furthermore, the above DNA sequences encoding the HF1-binding proteins, analogs or derivatives thereof, or the proteins, analogs and derivatives which they encode, may be used in a number of ways (see uses (i)-(ix) above) for the specific modulation of the HF1-associated effect on cells or the FAS-R effect on cells by way of modulation of the HF1 binding to FAS-R.

The isolation of the HF1-binding proteins, their identification and characterization may be carried out by any of the standard screening techniques used for isolating and identifying proteins, for example, the yeast two-hybrid method, affinity chromatography methods, and any of the other well-known standard procedures used for this purpose.

Other aspects and embodiments of the present invention are also provided as arising from the following detailed description of the invention.

It should be noted that, where used throughout, the following terms : "Modulation of the TNF-effect on cells"; "Modulation of the FAS-ligand effect on cells"; and "Modulation of the HF1 effect on cells" are understood to encompass *in vitro* as well as *in vivo* treatment.

Brief Description of the Drawings

Figs 1a-c depict schematically the partial and preliminary nucleotide sequence of cDNA clones encoding the p55IC and p75IC-binding proteins, wherein Fig. 1(a) is the

partial and preliminary sequence of clone 55.11 encoding the p55IC-binding protein 55.11; Fig. 1(b) is the partial and preliminary sequence of clone 75.3 encoding the p75IC-binding protein 75.3; and Fig. 1(c) is the partial and preliminary sequence of clone 75.16 encoding the p75IC-binding protein p75.16; all as described in Example 1.

Fig. 2 is a reproduction of a Western blot stained with anti-MBP polyclonal antiserum, showing the self association of the p55IC, the Western blot derived from an SDS-PAGE gel on which were electrophoresed the interacting bacterially-produced chimeric proteins p55IC-MBP and p55IC-GST (lanes 1-4) or the control interaction between the chimeric protein p55IC-MBP and GST alone (lanes 5-8), the interactions between the chimeric proteins (and control) being carried out on glutathion-agarose beads prior to SDS-PAGE, as described in Example 2.

Fig. 3 is a reproduction of phase contrast micrographs showing the cytotoxic effect of the full-length p55IC in HTta1 cells transfected with an expression vector encoding this p55IC (right panel); and the inhibition of this cytotoxic effect when expression of the vector is blocked by treating the cells with tetracycline (left panel), as described in Example 2.

Fig. 4 (A and B) depicts graphically the ligand independent triggering of a cytocidal effect in HeLa cells transfected with p55R or parts thereof, or with FAS-IC, wherein in Fig. 4A there is depicted the results with respect to the p55R or parts thereof and in Fig. 4B there is depicted the results with respect to the FAS-IC. In the left hand panels of both Fig. 4A and B there is depicted schematically the portion of the p55R or FAS-IC used in the transfections while the right hand panels depict graphically the experimental results, all as described in Example 2.

Fig. 5 A and B are reproductions of autoradiograms of SDS-PAGE gels (10% acrylamide) showing the interaction between HF1 (MORT1) and FAS-IC *in vitro*, wherein Fig. 5A shows a control autoradiogram of an immunoprecipitate of the proteins (from extracts of HeLa cells transfected with the FLAG-HF1 (FLAG-MORT1) fusion protein or with the luciferase cDNA (control), the

immunoprecipitation being performed with anti-FLAG antibody; and wherein Fig. 5B shows an autoradiogram of a representative gel performed to evaluate the *in vitro* interaction between HF1 and FAS-IC by way of assessing, autoradiographically, the binding of [^{35}S]-methionine-metabolically labeled HF1 produced in transfected HeLa cells as a fusion protein with the FLAG octapeptide (FLAG-MORT1) to GST, human and mouse GST-FAS-IC fusion protein (GST-huFAS-IC, GST-mFAS-IC) and GST-FAS-IC fusion proteins in which the FAS-IC contained an Ile to Ala replacement mutation at position 225 (GST-mFAS-IC I225A). The [^{35}S]labeled proteins of the HeLa cells including the labeled FLAG-MORT1 fusion protein having been first extracted were subjected to interaction with the various GST and GST-FAS-IC proteins (bound to glutathione beads) and then to SDS-PAGE. As controls in all of the interaction experiments, extracts of HeLa cells transfected with luciferase were subjected to interactions with the GST and GST-FAS-IC fusion proteins and SDS-PAGE. Figs. 5 A and B are also described in Example 3.

Fig. 6 A, B and C are reproductions of autoradiograms of SDS-PAGE gels (10% acrylamide) on which were separated various immunoprecipitates from transfected HeLa cells and which show the *in vivo* interaction of HF1 (MORT1) with FAS-IC. The HeLa cells were transfected with DNA constructs encoding : HF1-FLAG (FLAG-MORT1) fusion protein alone, HF1-FLAG fusion protein and the human FAS-R (FLAG-MORT1 + Fas/APO1) or human FAS-R alone (Fas/APO1) (Fig. 6A); or with HF1-FLAG fusion protein and the human p55R (FLAG-MORT1 + p55R) (Fig. 6B); or with HF1-FLAG fusion protein and a chimeric fusion protein between human FAS-R and p55-R in which the extracellular domain of the FAS-R was replaced with the corresponding region of the p55-R (FLAG-MORT1 + p55-FAS chimera) or the FAS-R-p55-R chimeric fusion protein alone (p55-Fas chimera) (Fig. 6C). In all cases the transfected cells were metabolically labeled with [^{35}S]cysteine (20 μCi/ml) and [^{35}S]methionine (40 μCi/ml), and were subjected to protein extraction. The protein extracts from the different transfected cells were

then immunoprecipitated with various antibodies being anti-FLAG, anti-FAS, anti-p75-R and anti-p55-R antibodies (αFLAG, αFAS, αp75-R and αp55-R in Figs. 6A-C) and subjected to SDS-PAGE. On the left side of Fig. 6A there is indicated the protein bands corresponding to FAS-R (Fas/APO1) and HF1-FLAG (FLAG-MORT1); between Figs. 6A and B are shown the relative positions of standard molecular weight markers (in kDa); and on the right hand side of Fig. 6C there is indicated the protein bands corresponding to p55R and the p55-FAS chimera. Figs. 6A-C are also described in Example 3.

Fig. 7 shows a reproduction of a Northern blot in which poly A^+ RNA (0.3 μg) from HeLa cells transfected was probed with HF1 cDNA, as described in Example 3.

Figs. 8 depicts schematically the preliminary nucleotide and deduced amino acid sequence of HF1, as described in Example 3, in which the 'death domain' is underlined as is a possible translation start site, i.e. the underlined methionine residue at position 49 (bold, underlined M). The asterisk indicates the translation stop codon (nucleotides 769-771). At the beginning and in the middle of each line are provided two numerals depicting the relative positions of the nucleotides and amino acids of the sequence with respect to the start of the sequence (5' end), in which the first numeral denotes the nucleotide and the second numeral denoted the amino acid.

Fig. 9 shows the results of experiments to determine the C-terminal end of HF1, wherein Fig. 9 is a reproduction of an autoradiogram of an SDS-PAGE gel (10% acrylamide) on which were separated various HF1-FLAG fusion products expressed in HeLa cells and metabolically labeled with ^{35}S-cysteine and ^{35}S-methionine followed by immunoprecipitation with either anti-FLAG monoclonal antibodies (M2) (lanes 2, 4 and 6) or as a control, anti-p75 TNF-R antibodies (#9) (lanes 1, 3 and 5), as described in Example 3.

Figs. 10 (A and B) depict graphically the ligand-independent triggering of cytocidal effects in cells transfected with HF1, wherein cell viability was determined either by the neutral red uptake assay (Fig. 10A), or for specifically determining the viability of cells that expressed the transfected DNA, by measuring the amounts of placental

alkaline phosphatase secreted into the medium (Fig. 10B). HeLa cells were transfected with tetracycline-controlled expression vectors encoding HF1 (MORT1), human FAS-IC, human p55-IC, or luciferase (control), and in all cases also with a cDNA encoding the secreted alkaline phosphatase, which permitted the evaluation of the effect of transient expression of these proteins on the viability of the cells. In both Figs. 9A and 9B the open graphs represent transfected cells grown in the presence of tetracycline (1 μg/ml, to block expression) and the closed graphs represent transfected cells grown in the absence of tetracycline. Figs. 9A and B are also described in Example 3.

Fig. 11 depicts schematically the partial and preliminary nucleotide sequence of a cDNA clone, called 'F2', which encodes a protein capable of binding to the p55IC and FAS-IC, as described in Example 3.

Fig. 12 depicts schematically the partial and preliminary nucleotide sequence of a cDNA clone, called F9, which encodes a protein capable of binding to the p55IC and FAS-IC, as described in Example 3.

Fig. 13 depicts schematically the partial and preliminary nucleotide sequence of a cDNA clone, called DD11, which encodes a protein capable of binding to the p55IC, especially the p55DD, and FAS-IC, as described in Example 3.

Detailed Description of the Invention

The present invention relates, in one aspect, to novel proteins which are capable of binding to the intracellular domain of receptors belonging to the TNF/NGF superfamily, such as TNF-Rs and FAS-R and hence are considered as mediators or modulators of this superfamily of receptors, e.g. of the TNF-Rs and FAS-R, having a role in, for example, the signaling process that is initiated by the binding of TNF to the TNF-R and FAS ligand to FAS-R. Examples of these proteins are those which bind to the intracellular domain of the p55 TNF-R (p55IC), such as the proteins designated herein as 55.1, 55.3 and 55.11 (Example 1) as well as those encoded by cDNA clones F2, F9, and DD11 (Example 3); those which bind to the intracellular domain of the p75 TNF-R (p75IC), such as the

proteins designated herein as 75.3 and 75.16 (Example 1); and those which bind to the intracellular domain of FAS-R (FAS-IC), such as the proteins encoded by cDNA clones HF1 (MORT-1), F2, F9 and DD11 (Example 3). Proteins 55.1 and 55.3 have been found to represent portions or fragments of the intracellular domain of the p55 TNF-R (p55IC); other proteins, 55.11, 75.3 and 75.16, represent proteins not described at all prior to filing of IL 109632 (75.3, 75.16) or those that have been described (55.11, see Khan et al., 1992) but whose function and other characteristics, particularly, the ability to bind to a TNF-R, were not described in any way (see Example 1, below). The new proteins encoded by cDNA clones HF1, F2, F9 and DD11 also represent proteins previously not described at all, i.e. their sequence is not in the 'GENEBANK' or 'PROTEIN BANK' data banks of DNA or amino acid sequences.

Thus, the present invention concerns the DNA sequences encoding these proteins and the proteins encoded by these sequences.

Moreover, the present invention also concerns the DNA sequences encoding biologically active analogs and derivatives of these proteins, and the analogs and derivatives encoded thereby. The preparation of such analogs and derivatives is by standard procedure (see for example, Sambrook et al., 1989) in which in the DNA sequences encoding these proteins, one or more codons may be deleted, added or substituted by another, to yield analogs having at least a one amino acid residue change with respect to the native protein. Acceptable analogs are those which retain at least the capability of binding to the intracellular domain of the TNF/NGF receptor superfamily, such as FAS-R or TNF-R, e.g. the p55IC, p75IC or FAS-IC, or which can mediate any other binding or enzymatic activity, e.g. analogs which bind the p55, p75IC or FAS-IC but which do not signal, i.e. do not bind to a further downstream receptor, protein or other factor, or do not catalyze a signal-dependent reaction. In such a way analogs can be produced which have a so-called dominant-negative effect, namely, an analog which is defective either in binding to the, for example, p55IC, p75IC or FAS-IC, or in subsequent signaling following such binding. Such analogs can be used, for example, to inhibit the TNF- or FAS-ligand- effect by competing with the natural IC-binding proteins. Likewise,

so-called dominant-positive analogs may be produced which would serve to enhance, for example, the TNF or FAS ligand effect. These would have the same or better IC-binding properties and the same or better signaling properties of the natural IC-binding proteins. Similarly, derivatives may be prepared by standard modifications of the side groups of one or more amino acid residues of the proteins, or by conjugation of the proteins to another molecule e.g. an antibody, enzyme, receptor, etc., as are well known in the art.

The new TNF-R and FAS-R intracellular domain - binding proteins, e.g. the proteins 55.1, 55.3, 55.11, 75.3, 75.16 as well as the proteins encoded by cDNA clones HF1, F2, F9 and DD11 (hereinafter, HF1, F2, F9 and DD11) have a number of possible uses, for example:

(i) They may be used to mimic or enhance the function of TNF or FAS-R ligand, in situations where an enhanced TNF or FAS-R ligand effect is desired such as in anti-tumor, anti-inflammatory or anti-HIV applications where the TNF-or FAS-R ligand- induced cytotoxicity is desired. In this case the proteins, e.g. those binding to the p55IC such as 5.1, 55.3, as well as F2, F9 and DD11, and the free p55IC itself (see below and Example 2), as well as the 'death domain' of the p55IC (p55DD - see IL 111125), which enhance the TNF effect; or proteins HF1, F2, F9 and DD11 as well as FAS-IC and FAS-DD which enhance the FAS-R ligand effect, i.e. cytotoxic effect, may be introduced to the cells by standard procedures known per se. For example, as the proteins are intracellular and it is desired that they be introduced only into the cells where the TNF or FAS-R ligand effect is wanted, a system for specific introduction of these proteins into the cells is necessary. One way of doing this is by creating a recombinant animal virus e.g. one derived from Vaccinia, to the DNA of which the following two genes will be introduced: the gene encoding a ligand that binds to cell surface proteins specifically expressed by the cells e.g. ones such as the AIDs (HIV) virus gp120 protein which binds specifically to some cells (CD4 lymphocytes and related leukemias) or any other ligand that binds specifically to cells carrying a TNF-R or FAS-R, such that the recombinant virus vector will be capable of binding such TNF-R- or FAS-R-

carrying cells; and the gene encoding the new intracellular domain-binding protein or the p55IC, p55DD, FAS-IC or FAS-DD protein. Thus, expression of the cell-surface-binding protein on the surface of the virus will target the virus specifically to the tumor cell or other TNF-R- or FAS-R- carrying cell, following which the intracellular domain-binding protein encoding sequence or p55IC, p55DD, FAS-IC or FAS-DD encoding sequence will be introduced into the cells via the virus, and once expressed in the cells will result in enhancement of the TNF or FAS-R ligand effect leading to the death of the tumor cells or other TNF-R- or FAS-R- carrying cells it is desired to kill. Construction of such recombinant animal virus is by standard procedures (see for example, Sambrook et al., 1989). Another possibility is to introduce the sequences of the new proteins or the p55IC, p55DD, FAS-IC or FAS-DD in the form of oligonucleotides which can be absorbed by the cells and expressed therein.

(ii) They may be used to inhibit the TNF or FAS-R ligand effect, e.g. in cases such as tissue damage in septic shock, graft-vs.-host rejection, or acute hepatitis, in which case it is desired to block the TNF-induced TNF-R or FAS-R ligand induced FAS-R intracellular signaling. In this situation it is possible, for example, to introduce into the cells, by standard procedures, oligonucleotides having the anti-sense coding sequence for these new proteins, or the anti-sense coding sequence for p55IC, p55DD, FAS-IC or FAS-DD, which would effectively block the translation of mRNAs encoding these proteins and thereby block their expression and lead to the inhibition of the TNF- or FAS-R ligand- effect.

Such oligonucleotides may be introduced into the cells using the above recombinant virus approach, the second sequence carried by the virus being the oligonucleotide sequence. Another possibility is to use antibodies specific for these proteins to inhibit their intracellular signaling activity. It is possible that these new proteins have an extracellular domain as well as an intracellular one, the latter which binds to the TNF-R or FAS-R binding domain, and thus antibodies

generated to their extracellular domains can be used to block their TNF- or FAS-R ligand- related functions.

Yet another way of inhibiting the TNF or FAS-R ligand effect is by the recently developed ribozyme approach. Ribozymes are catalytic RNA molecules that specifically cleave RNAs. Ribozymes may be engineered to cleave target RNAs of choice, e.g. the mRNAs encoding the new proteins of the invention or the mRNA encoding the p55IC, p55DD, FAS-IC or FAS-DD. Such ribozymes would have a sequence specific for the mRNA of choice and would be capable of interacting therewith (complementary binding) followed by cleavage of the mRNA, resulting in a decrease (or complete loss) in the expression of the protein it is desired to inhibit, the level of decreased expression being dependent upon the level of ribozyme expression in the target cell. To introduce ribozymes into the cells of choice (e.g. those carrying TNF-Rs or FAS-R) any suitable vector may be used, e.g. plasmid, animal virus (retrovirus) vectors, that are usually used for this purpose (see also (i) above, where the virus has, as second sequence, a cDNA encoding the ribozyme sequence of choice). Moreover, ribozymes can be constructed which have multiple targets (multi-target ribozymes) that can be used, for example, to inhibit the expression of one or more of the proteins of the invention and/or the p55IC, p55DD, FAS-IC or FAS-DD as well (For reviews, methods etc. concerning ribozymes see Chen et al., 1992; Zhao and Pick, 1993; Shore et al., 1993; Joseph and Burke, 1993; Shimayama et al., 1993; Cantor et al., 1993; Barinaga, 1993; Crisell et al., 1993 and Koizumi et al., 1993).

(iii) They may be used to isolate, identify and clone other proteins which are capable of binding to them, e.g. other proteins involved in the intracellular signaling process that are downstream of the TNF-R or FAS-R intracellular domain. In this situation, these options, namely, the DNA sequences encoding them may be used in the yeast two-hybrid system (see Example 1, below) in which the sequence of these proteins will be used as "baits" to isolate, clone and identify from cDNA or genomic DNA libraries other sequences ("preys") encoding proteins which can

bind to these new TNF-R or FAS-R intracellular domain-binding proteins. In the same way, it may also be determined whether the specific proteins of the present invention, namely, those which bind to the p55IC, p75IC, or FAS-IC, can bind to other receptors of the TNF/NGF superfamily of receptors. For example, it has recently been reported (Schwalb et al., 1993; Baens et al., 1993; Crowe et al., 1994) that there exist other TNF-Rs besides the p55 and p75 TNF-Rs. Accordingly, using the yeast two-hybrid system it may be specifically tested whether the proteins of the present invention are capable of specifically binding to these other TNF-Rs or other receptors of the TNF/NGF superfamily. Moreover, this approach may also be taken to determine whether the proteins of the present invention are capable of binding to other known receptors in whose activity they may have a functional role.

(iv) The new proteins may also be used to isolate, identify and clone other proteins of the same class i.e. those binding to TNF-R or FAS-R intracellular domains or to functionally related receptors, and involved in the intracellular signaling process. In this application the above noted yeast two-hybrid system may be used, or there may be used a recently developed (Wilks et al., 1989) system employing non-stringent southern hybridization followed by PCR cloning. In the Wilks et al. publication, there is described the identification and cloning of two putative protein-tyrosine kinases by application of non-stringent southern hybridization followed by cloning by PCR based on the known sequence of the kinase motif, a conceived kinase sequence. This approach may be used, in accordance with the present invention using the sequences of the new proteins to identify and clone those of related TNF-R, FAS-R or related receptor (TNF/NGF superfamily receptors) intracellular domain-binding proteins.

(v) Yet another approach to utilizing the new proteins of the invention is to use them in methods of affinity chromatography to isolate and identify other proteins or factors to which they are capable of binding, e.g. other receptors related to TNF-Rs (TNF/NGF receptor superfamily) or other proteins or factors involved in

the intracellular signaling process. In this application, the proteins of the present invention, may be individually attached to affinity chromatography matrices and then brought into contact with cell extracts or isolated proteins or factors suspected of being involved in the intracellular signaling process. Following the affinity chromatography procedure, the other proteins or factors which bind to the new proteins of the invention, can be eluted, isolated and characterized.

(vi) As noted above, the new proteins of the invention may also be used as immunogens (antigens) to produce specific antibodies thereto. These antibodies may also be used for the purposes of purification of the new proteins either from cell extracts or from transformed cell lines producing them. Further, these antibodies may be used for diagnostic purposes for identifying disorders related to abnormal functioning of the TNF or FAS-R ligand system, e.g. overactive or underactive TNF- or FAS-R ligand- induced cellular effects. Thus, should such disorders be related to a malfunctioning intracellular signaling system involving the new proteins, such antibodies would serve as an important diagnostic tool.

It should also be noted that the isolation, identification and characterization of the new proteins of the invention may be performed using any of the well known standard screening procedures. For example, one of these screening procedures, the yeast two-hybrid procedure as is set forth in the following examples (Examples 1 and 3), was used to identify the new proteins of the invention. Likewise as noted above and below, other procedures may be employed such as affinity chromatography, DNA hybridization procedures, etc. as are well known in the art, to isolate, identify and characterize the new proteins of the invention or to isolate, identify and characterize additional proteins, factors, receptors, etc. which are capable of binding to the new proteins of the invention or to the receptors belonging to the TNF/NGF family of receptors.

Furthermore, one of the above mentioned FAS-R intracellular domain-binding proteins of the invention is the HF1 protein (see Example 3), represents another aspect of the invention. Amongst the characteristics of HF1 is its ability to bind to the FAS-IC and

also its ability to self-associate. HF1 is also capable of activating cell cytotoxicity on its own, an activity related to its self-association ability. It appears (see Example 3) that the part of HF1 which binds to the FAS-IC is distinct from the part of HF1 which is involved in its self-association. HF1 may also have other activities which may be a function of the above noted distinct parts of the HF1 molecule or other parts of the molecule or combinations of any of these parts. These other activities may be enzymatic or related to binding other proteins (e.g. HF1-binding proteins or other receptors, factors, etc.). Thus, HF1 may be used in the above methods for modulation of FAS-R-ligand effects or its own HF1-mediated cellular effects, or it may be used in the modulation of other cellular signaling processes related to other receptors, factors, etc.

As mentioned hereinabove, biologically active analogs, derivatives and active fragments of HF1 may be prepared by standard recombinant DNA procedures by using the DNA sequence encoding HF1 (see Fig. 8) and manipulating it to delete, add or replace one or more codons to yield analogs having at least a one amino acid residue change with respect to the native protein. Acceptable analogs are those which retain at least the capability of binding to the FAS-IC, or which can mediate any other binding or enzymatic activity, e.g. analogs which bind the FAS-IC but which do not signal, i.e. do not bind to a further downstream receptor, protein or other factor, or do not catalyze a signal-dependent reaction. In such a way analogs can be produced which have a so-called dominant-negative effect, namely, an analog which is defective either in binding to the FAS-IC or in subsequent signaling following such binding. Such analogs can be used, for example, to inhibit the FAS-IC effect by competing with the natural FAS-IC-binding proteins. Likewise, so-called dominant positive analogs may be produced which would serve to enhance, for example, the FAS-ligand effect. These would have the same or better FAS-IC binding properties and the same or better signaling properties of the natural FAS-IC-binding proteins. Similarly, HF1 derivatives may be prepared by standard modifications of the side groups of one or more amino acid residues of the protein or by conjugation of the protein to another molecule, e.g. antibody, enzyme, receptor, etc. as are well known in the art.

Accordingly, the HF1 protein, analogs, derivatives and active fragments thereof may be used as set forth hereinabove in a number of ways (see uses (i)-(vi) above). Thus, HF1, its analogs or derivatives may be used to mimic or enhance the function of FAS-R ligand; may be used to inhibit the FAS-R ligand effect; may be used to isolate, identify and clone other proteins which are capable of binding to it; may be used to isolate, identify and clone other proteins of the same class, i.e. those binding to FAS-IC; may be used in methods of affinity chromatography to isolate and identify other proteins or factors; and may be used to generate specific anti-HF1 antibodies, or as set forth in detail in (i)-(vi) above where it is to be understood that in this aspect these uses are directed to use of the HF1 protein, its analogs or its derivatives or DNA sequences coding therefor, for mediating or modulating the activity of the FAS-R receptor (or mediating or modulating the FAS ligand-effect), or for isolating and identifying HF1-binding proteins.

More specifically, by this aspect of the invention, the HF1 encoding DNA molecule itself and mutations thereof (i.e. encoding analogs or active fractions of HF1) can be used for gene therapy (i.e. by the ways set forth in uses (i) and (ii) above) for modulating the activity of the FAS-R (or modulating or mediating the FAS ligand-effect on cells). Moreover, as HF1 also has a cytotoxic effect on cells, these HF1 or mutant HF1 encoding DNA molecules may also be used for gene therapy for modulating the HF1 effect in cells (also by way of the uses (i) and (ii) above). In these gene therapy applications, the HF1, analogs or derivatives may be used in three ways :

(a) the whole HF1 protein, its analogs, derivatives or active fragments which have both FAS-IC and HF1-binding ability (i.e. contain the two regions of HF1, one of which is involved in binding to FAS-IC and the other which is involved in the self-association of HF1) may be used to modulate FAS-R and HF1-associated effects;

(b) the part of HF1, and analogs, derivatives, and active fragments, etc. of this part which binds to the FAS-IC may be used for inducing a 'dominant negative' effect on FAS-IC, i.e. inhibition of FAS-R-mediated cellular effects, or may be used for inducing a 'gain of function' effect on FAS-IC, i.e. enhancement of the FAS-R-mediated cellular effects; and

(c) the part of HF1 and analogs, derivatives and active fractions of this part, which binds specifically to HF1 may be used for induction of 'dominant negative' or 'gain of function' effects on HF1, i.e. either inhibition or enhancement of HF1-associated cellular effects.

As set forth in uses (iii)-(v) above, the DNA sequence encoding HF1 or the HF1 protein may be used to isolate, identify and characterize other proteins which are capable of specifically binding to HF1, i.e. the HF1-binding proteins of the invention. For example, the HF1 encoding DNA molecule may be used with the yeast two-hybrid technique to isolate, identify and characterize HF1-binding proteins; or the HF1 protein may be used by affinity chromatography procedures to isolate such HF1-binding proteins. Furthermore, any of the other well known standard methods of the art for isolating, identifying and characterizing proteins may be employed for isolating, identifying and characterizing the HF1-binding proteins, in which methods the HF1, or analogs, derivatives or parts thereof or DNA sequences encoding any of the aforementioned may be used, where appropriate, to isolate, identify and subsequently to characterize the HF1-binding proteins.

The HF1-binding proteins may be produced as mentioned hereinabove by any of the standard recombinant DNA procedures, as may be produced analogs, derivatives or active fragments of these HF1-binding proteins.

The HF1-binding proteins may be utilized, along the lines set forth in the uses (i) and (ii) above to mimic or enhance the HF1-associated cellular effects or to inhibit the HF1-associated cellular effects. Further, the HF1-binding proteins may also be used, where applicable, to enhance or to inhibit, indirectly, the FAS-R ligand-induced effects by way of enhancing or inhibiting the activity of HF1.

As set forth in use (vi) above, the HF1 protein or HF1-binding proteins may be used to generate antibodies specific to HF1 or HF1-binding proteins. These antibodies or fragments thereof may be used as set forth hereinbelow in detail, to be understood that in these applications the antibodies or fragments thereof are those specific for HF1 or HF1-binding proteins.

Moreover, the HF1-binding proteins may themselves be employed, in an analogous fashion to the way in which HF1 was used as noted above and below, to isolate, identify and characterize other proteins, factors, etc. which are capable of binding to the HF1-binding proteins and which may represent factors involved further downstream in the associated signaling process, or which may be able to bind HF1 or FAS-R or other receptors or factors and thereby also being involved in the associated signaling process, or which may have cell cytotoxic effects on their own (like HF1) and hence would represent proteins involved in a distinct signaling process. In this regard, it should also be mentioned that the HF1-binding proteins may also have enzymatic or cell cytotoxic functions and thus may also be involved in distinct signaling processes, permitting the use of the HF1-binding proteins and/or HF1 and/or further proteins, factors, receptors, etc. which bind to any of HF1 or HF1-binding proteins in methods for modulating these HF1-binding protein-associated enzymatic or cell cytotoxic functions.

As regards the antibodies mentioned herein throughout, the term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies, anti-idiotypic (anti-Id) antibodies to antibodies that can be labeled in soluble or bound form, as well as fragments thereof provided by any known technique, such as, but not limited to enzymatic cleavage, peptide synthesis or recombinant techniques.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen. A monoclonal antibody contains a substantially homogeneous population of antibodies specific to antigens, which populations contains substantially similar epitope binding sites. MAbs may be obtained by methods known to those skilled in the art. See, for example Kohler and Milstein, Nature, 256:495-497 (1975); U.S. Patent No. 4,376,110; Ausubel et al., eds., Harlow and Lane ANTIBODIES : A LABORATORY MANUAL, Cold Spring Harbor Laboratory (1988); and Colligan et al., eds., Current Protocols in Immunology, Greene publishing Assoc. and Wiley Interscience N.Y., (1992, 1993), the contents of which references are incorporated entirely herein by reference. Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, GILD and any subclass thereof. A hybridoma producing a mAb of the

present invention may be cultivated *in vitro*, *in situ* or *in vivo*. Production of high titers of mAbs *in vivo* or *in situ* makes this the presently preferred method of production.

Chimeric antibodies are molecules different portions of which are derived from different animal species, such as those having the variable region derived from a murine mAb and a human immunoglobulin constant region. Chimeric antibodies are primarily used to reduce immunogenicity in application and to increase yields in production, for example, where murine mAbs have higher yields from hybridomas but higher immunogenicity in humans, such that human/murine chimeric mAbs are used. Chimeric antibodies and methods for their production are known in the art (Cabilly et al., *Proc. Natl. Acad. Sci. USA* 81:3273-3277 (1984); Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984); Boulianne et al., *Nature* 312:643-646 (1984); Cabilly et al., European Patent Application 125023 (published November 14, 1984); Neuberger et al., *Nature* 314:268-270 (1985); Taniguchi et al., European Patent Application 171496 (published February 19, 1985); Morrison et al., European Patent Application 173494 (published March 5, 1986); Neuberger et al., PCT Application WO 8601533, (published March 13, 1986); Kudo et al., European Patent Application 184187 (published June 11, 1986); Sahagan et al., *J. Immunol.* 137:1066-1074 (1986); Robinson et al., International Patent Application No. WO8702671 (published May 7, 1987); Liu et al., *Proc. Natl. Acad. Sci USA* 84:3439-3443 (1987); Sun et al., *Proc. Natl. Acad. Sci USA* 84:214-218 (1987); Better et al., *Science* 240:1041-1043 (1988); and Harlow and Lane, ANTIBODIES :A LABORATORY MANUAL, supra. These references are entirely incorporated herein by reference.

An anti-idiotypic (anti-Id) antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An Id antibody can be prepared by immunizing an animal of the same species and genetic type (e.g. mouse strain) as the source of the mAb with the mAb to which an anti-Id is being prepared. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the

anti-Id antibody). See, for example, U.S. Patent No. 4,699,880, which is herein entirely incorporated by reference.

The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. The anti-anti-Id may be epitopically identical to the original mAb which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of a mAb, it is possible to identify other clones expressing antibodies of identical specificity.

Accordingly, mAbs generated against the IC-binding proteins, analogs or derivatives thereof, of the present invention or the p55IC, p55DD, FAS-IC, FAS-DD, analogs or derivatives thereof may be used to induce anti-Id antibodies in suitable animals, such as BALB/c mice. Spleen cells from such immunized mice are used to produce anti-Id hybridomas secreting anti-Id mAbs. Further, the anti-Id mAbs can be coupled to a carrier such as keyhole limpet hemocyanin (KLH) and used to immunize additional BALB/c mice. Sera from these mice will contain anti-anti-Id antibodies that have the binding properties of the original mAb specific for an epitope of the above IC-binding proteins, analogs or derivatives or p55IC, p55DD, FAS-IC or FAS-DD, analogs or derivatives.

The anti-Id mAbs thus have their own idiotypic epitopes, or "idiotopes" structurally similar to the epitope being evaluated, such as GRB protein-α.

The term "antibody" is also meant to include both intact molecules as well as fragments thereof, such as, for example, Fab and $F(ab')_2$, which are capable of binding antigen. Fab and $F(ab')_2$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983)).

It will be appreciated that Fab and $F(ab')_2$ and other fragments of the antibodies useful in the present invention may be used for the detection and quantitation of the IC-binding proteins or p55IC, p55DD, FAS-IC or FAS-DD according to the methods disclosed herein for intact antibody molecules. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments).

An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody. The term "epitope" is meant to refer to that portion of any molecule capable of being bound by an antibody which can also be recognized by that antibody. Epitopes or "antigenic determinants" usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three dimensional structural characteristics as well as specific charge characteristics.

An "antigen" is a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen may have one or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

The antibodies, including fragments of antibodies, useful in the present invention may be used to quantitatively or qualitatively detect the IC-binding proteins or p55IC, p55DD, FAS-IC, FAS-DD in a sample or to detect presence of cells which express the IC-binding proteins of the present invention or the p55IC, p55DD, FAS-IC, FAS-DD proteins. This can be accomplished by immunofluorescence techniques employing a fluorescently labeled antibody (see below) coupled with light microscopic, flow cytometric, or fluorometric detection.

The antibodies (or fragments thereof) useful in the present invention may be employed histologically, as in immunofluorescence or immunoelectron microscopy, for *in situ* detection of IC-binding proteins of the present invention or the p55IC, p55DD, FAS-IC, FAS-DD. *In situ* detection may be accomplished by removing a histological specimen from a patient, and providing the labeled antibody of the present invention to such a specimen. The antibody (or fragment) is preferably provided by applying or by overlaying the labeled antibody (or fragment) to a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the IC-binding proteins or the p55IC, p55DD, FAS-IC, FAS-DD, but also its distribution on the examined tissue.

Using the present invention, those of ordinary skill will readily perceive that any of wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

Such assays for IC-binding proteins of the present invention or the p55IC, p55DD, FAS-IC, FAS-DD, typically comprises incubating a biological sample, such as a biological fluid, a tissue extract, freshly harvested cells such as lymphocytes or leukocytes, or cells which have been incubated in tissue culture, in the presence of a detectably labeled antibody capably of identifying the IC-binding proteins or the p55IC, p55DD, FAS-IC, FAS-DD, and detecting the antibody by any of a number of techniques well known in the art.

The biological sample may be treated with a solid phase support or carrier such as nitrocellulose, or other solid support or carrier which is capable of immobilizing cells, cell particles or soluble proteins. The support or carrier may then be washed with suitable buffers followed by treatment with a detectably labeled antibody in accordance with the present invention, as noted above. The solid phase support or carrier may then be washed with the buffer a second time to remove unbound antibody. The amount of bound label on said solid support or carrier may then be detected by conventional means.

By "solid phase support", "solid phase carrier", "solid support", "solid carrier", "support" or "carrier" is intended any support or carrier capable of binding antigen or antibodies. Well-known supports or carriers, include glass, polystyrene, polypropylene, polyethylene, dextran, nylon amylases, natural and modified celluloses, polyacrylamides, gabbros and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support or carrier configuration may be spherical, as in a bead, cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports or carriers include polystyrene beads. Those skilled in the art will know

may other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

The binding activity of a given lot of antibody, of the invention as noted above, may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

Other such steps as washing, stirring, shaking, filtering and the like may be added to the assays as is customary or necessary for the particular situation.

One of the ways in which an antibody in accordance with the present invention can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA). This enzyme, in turn, when later exposed to an appropriate substrate, will react with the substrate in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or by visual means. Enzymes which can be used detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomeras, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. The detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

Detection may be accomplished using any of a variety of other immunoassays. For example, by radioactivity labeling the antibodies or antibody fragments, it is possible to detect R-PTPase through the use of a radioimmunoassay (RIA). A good description of RIA may be found in Laboratory Techniques and Biochemistry in Molecular Biology, by Work, T.S. et al., North Holland Publishing Company, NY (1978) with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard, T., incorporated by reference herein. The radioactive isotope can

be detected by such means as the use of a γ counter or a scintillation counter or by autoradiography.

It is also possible to label an antibody in accordance with the present invention with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wavelength, its presence can be then detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrine, pycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}E, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriamine pentaacetic acid (ETPA).

The antibody can also be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

An antibody molecule of the present invention may be adapted for utilization in an immunometric assay, also known as a "two-site" or "sandwich" assay. In a typical immunometric assay, a quantity of unlabeled antibody (or fragment of antibody) is bound to a solid support or carrier and a quantity of detectably labeled soluble antibody is added to permit detection and/or quantitation of the ternary complex formed between solid-phase antibody, antigen, and labeled antibody.

Typical, and preferred, immunometric assays include "forward" assays in which the antibody bound to the solid phase is first contacted with the sample being tested to extract

the antigen from the sample by formation of a binary solid phase antibody-antigen complex. After a suitable incubation period, the solid support or carrier is washed to remove the residue of the fluid sample, including unreacted antigen, if any, and the contacted with the solution containing an unknown quantity of labeled antibody (which functions as a "reporter molecule"). After a second incubation period to permit the labeled antibody to complex with the antigen bound to the solid support or carrier through the unlabeled antibody, the solid support or carrier is washed a second time to remove the unreacted labeled antibody.

In another type of "sandwich" assay, which may also be useful with the antigens of the present invention, the so-called "simultaneous" and "reverse" assays are used. A simultaneous assay involves a single incubation step as the antibody bound to the solid support or carrier and labeled antibody are both added to the sample being tested at the same time. After the incubation is completed, the solid support or carrier is washed to remove the residue of fluid sample and uncomplexed labeled antibody. The presence of labeled antibody associated with the solid support or carrier is then determined as it would be in a conventional "forward" sandwich assay.

In the "reverse" assay, stepwise addition first of a solution of labeled antibody to the fluid sample followed by the addition of unlabeled antibody bound to a solid support or carrier after a suitable incubation period is utilized. After a second incubation, the solid phase is washed in conventional fashion to free it of the residue of the sample being tested and the solution of unreacted labeled antibody. The determination of labeled antibody associated with a solid support or carrier is then determined as in the "simultaneous" and "forward" assays.

The new proteins of the invention (including HF1 and HF1-binding proteins) once isolated, identified and characterized by any of the standard screening procedures, for example, the yeast two-hybrid method, affinity chromatography, and any other well known method known in the art, may then be produced by any standard recombinant DNA procedure (see for example, Sambrook, et al., 1989) in which suitable eukaryotic or prokaryotic host cells are transformed by appropriate eukaryotic or prokaryotic vectors

containing the sequences encoding for the proteins. Accordingly, the present invention also concerns such expression vectors and transformed hosts for the production of the proteins of the invention. As mentioned above, these proteins also include their biologically active analogs and derivatives, and thus the vectors encoding them also include vectors encoding analogs of these proteins, and the transformed hosts include those producing such analogs. The derivatives of these proteins are the derivatives produced by standard modification of the proteins or their analogs, produced by the transformed hosts.

In another aspect, the invention relates to the use of the free intracellular domain of the p55 TNR-R (p55IC) or FAS-R (FAS-IC) or their so-called 'death domains' (p55DD or FAS-DD, respectively) as an agent for enhancing the TNF or FAS-R ligand effect on cells, on its own (see Example 2). Where it is desired to introduce a TNF- or FAS-R-ligand-induced cytotoxic effect in cells, e.g. cancer cells or HIV-infected cells, the p55IC, p55DD, FAS-IC or FAS-DD can be introduced into such cells using the above noted (see (i) above) recombinant animal virus (e.g. vaccinia) approach. Here too, the native p55IC, p55DD, FAS-IC or FAS-DD, biologically active analogs and derivatives or fragments may be used, all of which can be prepared as noted above.

Likewise, the present invention also relates to the specific blocking of the TNF-effect or FAS-R ligand-effect by blocking the activity of the p55IC, p55DD, FAS-IC or FAS-DD, e.g. anti-sense oligonucleotides may be introduced into the cells to block the expression of the p55IC, p55DD, FAS-IC or FAS-DD.

The present invention also relates to pharmaceutical compositions comprising recombinant animal virus vectors encoding the TNF-R or FAS-R intracellular domain binding proteins (including the p55IC, p55DD, FAS-IC and FAS-DD), which vector also encodes a virus surface protein capable of binding specific target cell (e.g. cancer cells) surface proteins to direct the insertion of the intracellular domain binding protein sequences into the cells. Other aspects of the invention will be apparent from the following examples.

The invention will now be described in more detail in the following non-limiting examples and the accompanying drawings :

EXAMPLE 1

Cloning and isolation of proteins which bind to the intracellular domains of the p55 and p75 TNF receptors

This example is set forth also in IL 109632, and is included herein because of its detailed description of the method for isolating and identifying other TNF/NGF receptor superfamily intracellular domain-binding proteins, e.g. those set forth in Example 3 below. It should be noted, however, that some additions have been made in this example to incorporate new experimental data, e.g. an update of the hereinbelow designated protein 55.11 (see Fig. 1 (a)).

To isolate proteins interacting with the intracellular domains of the p55 and p75 TNF receptors (p55IC and p75 IC), the yeast two-hybrid system was used (Fields and Song, 1989). Briefly, this two-hybrid system is a yeast-based genetic assay to detect specific protein-protein interactions *in vivo* by restoration of a eukaryotic transcriptional activator such as GAL4 that has two separate domains, a DNA binding and an activation domain, which domains when expressed and bound together to form a restored GAL4 protein, is capable of binding to an upstream activating sequence which in turn activates a promoter that controls the expression of a reporter gene, such as lacZ or HIS3, the expression of which is readily observed in the cultured cells. In this system the genes for the candidate interacting proteins are cloned into separate expression vectors. In one expression vector the sequence of the one candidate protein is cloned in phase with the sequence of the GAL4 DNA-binding domain to generate a hybrid protein with the GAL4 DNA-binding domain, and in the other vector the sequence of the second candidate protein is cloned in phase with the sequence of the GAL4 activation domain to generate a hybrid protein with the GAL4-activation domain. The two hybrid vectors are then co-transformed into a yeast host strain having a lacZ or HIS3 reporter gene under the control of upstream GAL4 binding sites. Only those transformed host cells (cotransformants) in which the two hybrid proteins are expressed and are capable of interacting with each other, will be capable of expression of the reporter gene. In the case of the lacZ reporter gene, host cells expressing this gene will become blue in color when X-gal is added to the

cultures. Hence, blue colonies are indicative of the fact that the two cloned candidate proteins are capable of interacting with each other.

Using this two-hybrid system, the intracellular domains p55IC and p75IC were cloned, separately, into the vector pGBT9 (carrying the GAL4 DNA-binding sequence, provided by CLONTECH, USA, see below), to create fusion proteins with the GAL4 DNA-binding domain (similarly, the intracellular domain, FAS-IC and a portion of the 55IC, namely, the 55DD were also cloned into pGBT9 and used to isolate other IC-binding proteins, see Example 3 below). For the cloning of p55IC and p75IC into pGBT9, clones encoding the full-length cDNA sequences of p55 TNF-R (Schall et al., 1990) and p75 TNF-R (Smith et al., 1990) were used from which the intracellular domains (IC) were excised as follows : p55IC was excised using the enzymes EcoRI and SalI, the EcoRI-SalI fragment containing the p55IC sequence was then isolated by standard procedures and inserted into the pGBT9 vector opened, in its multiple cloning site region (MCS), with EcoRI and SalI. p75 IC was excised using the enzymes BspHI and SalI, the BspHI-SalI fragment containing the p75 IC sequence was then isolated by standard procedures and filled-in with the Klenow enzyme to generate a fragment which could be inserted into the pGBT9 vector opened with SmaI and SalI.

The above hybrid (chimeric) vectors were then cotransfected (separately, one cotransfection with the p55IC hybrid and one with the p75 IC hybrid vector) together with a cDNA library from human HeLa cells cloned into the pGAD GH vector, bearing the GAL4 activating domain, into the HF7c yeast host strain (all the above-noted vectors, pGBT9 and pGAD GH carrying the HeLa cell cDNA library, and the yeast strain were purchased from Clontech Laboratories, Inc., USA, as a part of MATCHMAKER Two-Hybrid System, #PT1265-1). The co-transfected yeasts were selected for their ability to grow in medium lacking Histidine (His^- medium), growing colonies being indicative of positive transformants. The selected yeast clones were then tested for their ability to express the lacZ gene, i.e. for their LAC Z activity, and this by adding X-gal to the culture medium, which is catabolized to form a blue colored product by β-galactosidase, the enzyme encoded by the lacZ gene. Thus, blue colonies are indicative of an active lacZ

gene. For activity of the lacZ gene, it is necessary that the GAL4 transcription activator be present in an active form in the transformed clones, namely that the GAL4 DNA-binding domain encoded by one of the above hybrid vectors be combined properly with the GAL4 activation domain encoded by the other hybrid vector. Such a combination is only possible if the two proteins fused to each of the GAL4 domains are capable of stably interacting (binding) to each other. Thus, the His^+ and blue (LAC Z^+) colonies that were isolated are colonies which have been cotransfected with a vector encoding p55IC and a vector encoding a protein product of human HeLa cell origin that is capable of binding stably to p55 IC; or which have been transfected with a vector encoding p75IC and a vector encoding a protein product of human HeLa cell origin that is capable of binding stably to p75 IC.

The plasmid DNA from the above His^+, LAC Z^+ yeast colonies was isolated and electroporated into E. coli strainHB101 by standard procedures followed by selection of Leu^+ and Ampicillin resistant transformants, these transformants being the ones carrying the hybrid pGAD GH vector which has both the Amp^R and Leu^2 coding sequences. Such transformants therefore are clones carrying the sequences encoding newly identified proteins capable of binding to the p55IC or p75IC. Plasmid DNA was then isolated from these transformed E. coli and retested by :

(a) retransforming them with the original intracellular domain hybrid plasmids (hybrid pGTB9 carrying either the p55IC or p75IC sequences) into yeast strain HF7 as set forth hereinabove. As controls, vectors carrying irrelevant protein encoding sequences, e.g. pACT-lamin or pGBT9 alone were used for cotransformation with the p55IC-binding protein or p75IC-binding protein encoding plasmids. The cotransformed yeasts were then tested for growth on His^- medium alone, or with different levels of 3-aminotriazole; and

(b) retransforming the plasmid DNA and original intracellular domain hybrid plasmids and control plasmids described in (a) into yeast host cells of strain SFY526 and determining the LAC Z^+ activity (effectivity of β-gal formation, i.e. blue color formation).

The results of the above tests revealed that the pattern of growth of colonies in His^- medium was identical to the pattern of LAC Z activity, as assessed by the color of the

colony, i.e. His^+ colonies were also LAC Z^+. Further, the LAC Z activity in liquid culture (preferred culture conditions) was assessed after transfection of the GAL4 DNA-binding and activation-domain hybrids into the SFY526 yeast hosts which have a better LAC Z inducibility with the GAL4 transcription activator than that of the HF-7 yeast host cells.

The results of the above co-transfections are set forth in Table 1 below, from which it is apparent that a number of proteins were found that were capable of binding to the p55IC or the p75IC, namely, the proteins designated 55.11, which binds to the p55IC; and 75.3 and 75.16 which bind to the p75IC. All of these p55IC- and p75IC-binding proteins are authentic human proteins all encoded by cDNA sequences originating from the HeLa cell cDNA library, which were fused to the GAL4 activation-domain sequence in the plasmid pGAD GH in the above yeast two-hybrid analysis system.

Interestingly, it was also found that fragments of the p55IC, itself, namely, the proteins designated 55.1 and 55.3 were capable of binding to p55IC. These are discussed also in Example 2 below.

TABLE 1

SUMMARY OF THE CHARACTERISTICS OF SOME OF THE cDNA CLONES (SEE ALSO EXAMPLE 3) ISOLATED BY THE TWO-HYBRIDSYSTEM APPROACH

DNA-binding domain hybrid	Activation-domain hybrid	Colony color	Lac Z activity in liquid culture assay
pGBT9-IC55	---	white	0.00
pGBT9-IC55	55.1	blue	0.65
pGBT9-IC55	55.3	blue	0.04
---	55.1	white	0.00
---	55.3	white	0.00
pACT-Lamin	55.1	white	0.00
pACT-Lamin	55.3	white	0.00
pGBT9	55.1	white	0.00
pGBT9	55.3	white	0.00
pGBT9-IC55	55.11	blue	ND
---	55.11	white	ND
pACT-Lamin	55.11	white	ND
pGBT9	55.11	white	ND
pGBT9-IC75	75.3	blue	ND
pGBT9-IC75	---	white	ND
---	75.3	white	ND
pACT-Lamin	75.3	white	ND

pGBT9	75.3	white	ND
pGBT9-IC75	75.16	blue	ND
---	75.16	white	ND
pACT-Lamin	75.16	white	ND
pGBT9	75.16	white	ND

In the above Table 1, the plasmids and hybrid encoding the GAL4 DNA-binding domain and GAL4 activation domain are as follows :

DNA-binding domain hybrids :

pGBT9-IC55 : full-length intracellular domain of the p55-TNF-R (p55IC)

pACT-Lamin : irrelevant protein - lamin.

pGBT9 : vector alone

pGBT9-IC75 : full-length intracellular domain of the p75-TNF-R (p75IC)

Activation-domain hybrid :

55.1 and 55.3 correspond to fragments of the intracellular domain of the p55-TNF-R.

55.11 : is the novel protein associating with the p55-TNF-R

75.3 and 75.16 are the novel proteins associating with the p75-TNF-R.

The above noted cloned cDNAs encoding the novel p55IC- and p75IC- binding proteins, 55.11, 75.3 and 75.16, were then sequenced using standard DNA sequencing procedures. The partial sequence of all of these protein-encoding sequences is set forth in Figs. 1 a-c, where Fig. 1(a) depicts an updated version (i.e. post-IL109632) of the partial sequence of the cDNA encoding protein 55.11; Fig. 1(b) depicts the partial sequence of the cDNA encoding protein 75.3; and Fig. 1(c) depicts the partial sequence of the cDNA encoding protein 75.16.

It should be noted, however, that the partial sequence of the cDNA encoding the 55.11 protein has also been reported by Khan et al. (1992), in a study of human brain cDNA sequences, which study was directed at the establishment of a new rapid and accurate method for the sequencing and physical and genetic mapping of human brain cDNAs. However, Khan et al. did not provide any information as regards the function or any other characteristics of the protein encoded by the 55.11 cDNA sequence, such functional or other analysis not being the intention of Khan et al. in their study.

The above cDNA encoding protein 55.11 was then subjected to Northern blot analysis, in which the 55.11 cDNA was shown to hybridize to a mRNA transcript having a size of about 3kb, expressed in the following cell cultures: HeLa, Alexander (liver) and CEM (T cells).

EXAMPLE 2

Self-association ability of the intracellular domain of the p55 TNF receptor (p55IC) and its capability to cause cell death

This example is also from IL 109632 and is maintained herein because of its description of the self-associating capability of the p55IC and the ability of the p55IC to induce, in a ligand-independent fashion, cell cytotoxic effects. It should however be noted that some additions have been made to this example in view of new experimental data.

As set forth in Example 1 above, it was discovered that the intracellular domain of p55 TNF-R (p55IC) is capable of binding to itself, and further that fragments of p55IC, namely proteins 55.1 and 55.3, are also capable of binding to p55IC.

It is known that the binding of TNF to p55 TNF-R leads to a cytocidal effect on the cells carrying this receptor. Further, antibodies against the extracellular domain of this receptor can themselves trigger this effect, in correlation with the effectivity of receptor cross-linking by them.

In addition, mutational studies (Tartaglia et al., (1993); Brakebusch et al., (1992)) showed that the function of the p55-R depends on the integrity of its intracellular domain. It was therefore suggested that the initiation of signaling for the cytocidal effect of TNF occurs as a consequence of association of two or more intracellular domains of the p55-R (p55-IC), imposed by receptor aggregation. The results in accordance with the present invention provide further evidence for this notion, showing that expression of the intracellular domain of the p55-R within cells, without the transmembrane or intracellular domain, triggers their death. Such free intracellular domains of the p55-R are shown to self associate, which probably accounts for their ability to function independently of TNF. The fact that the signaling by the full length p55-R does depend on TNF stimulation is

suggested to reflect activiti(es) of the transmembrane or extracellular domain of the receptor which decrease or prevent this self association.

The ability of the intracellular domain of the p55-R (p55-IC) to self associate was found serendipitously, in the attempts to clone effector proteins which interact with this receptor (see Example 1 above). We applied for that purpose the above mentioned "two hybrid" technique. In addition to the novel protein, 55.11 found to associate (bind) to the p55IC, it was also found that three other cloned HeLa cell cDNAs contained cDNA sequences encoding for parts of the intracellular domain of the p55-R, implying that the p55-IC is capable of self-association. Two of these clones were identical, containing an insert which encodes for amino acids 328-426 (designated as clone 55.1 encoding protein fragment 55.1 of the p55IC). The third contained a longer insert, encoding for amino acids 277-426 (designated as clone 55.3 encoding protein fragment 55.3 of the p55IC).

In addition, we assessed the in vitro interaction between two bacterially produced chimeras of the p55IC, one, in which it was fused to the maltose binding protein (MBP) and the other in which is was fused to the glutathione-S-transferase (GST). These chimeras were constructed, cloned and expressed by standard methods. Following their expression, the assessment of the self-interaction of the p55-R intracellular domain (p55IC) by determining the interaction of the above bacterially-produced chimeric proteins GST-IC55 (Mr - 51kD) and MBP-IC55 (Mr - 67 kD) with each other. Equal amounts of the GST-IC55 chimera (samples of lanes 1-4 in Fig. 2) or GST alone (samples of lanes 5-8 in Fig. 2) were bound to glutathion-agarose beads (Sigma) and were then incubated with the same amount of MBP-IC55 fusion protein in one of the following buffer solutions :

(i) buffer I (20mM Tris-HCl, pH 7.5, 100mM KCl, 2mM $CaCl_2$, 2mM $MgCl_2$, 5mM DTT, 0.2% Triton X100, 0.5mM PMSF, 5% Glycerol). This was done for the samples of Lanes 1 and 5 of Fig. 2.

(ii) buffer I containing 5mM EDTA instead of $MgCl_2$. This was done for the samples of Lanes 2 and 6 of Fig. 2.

(iii) buffer I containing 250mM instead of 100mM KCl. This was done for the samples of Lanes 3 and 7 of Fig. 2.

(iv) buffer I containing 400mM instead of 100mM KCl. This was done for the samples of Lanes 4 and 8 of Fig. 2.

After incubation with rotation for 2h at 4°C, the beads were washed with the same buffers and then boiled in SDS-PAGE buffer followed by electrophoresis by PAGE. The proteins on the gel were then Western blotted to a nitrocellulose membrane which was then stained with polyclonal antiserum against MBP. A reproduction of this stained Western blot is shown in Fig. 2, the samples in lanes 1-8 being those noted above.

From Fig. 2 it is apparent that the p55IC-MBP chimera bind to the p55IC-GST chimeria (lanes 1-4) independently of divalent cations and even at a rather high salt concentration (0.4M KCl). Thus, it is concluded that the p55IC is able to avidly self-associate.

To evaluate the functional implications of the propensity of the p55-IC to self associate, we attempted to express the p55-IC within the cytoplasm of cells which are sensitive to the cytocidal effect of TNF. Considering the possibility that the p55-IC will turn to be cytotoxic, we chose to express it in an inducible manner, using the recently developed, tightly regulated tetracycline-controlled mammalian expression system (Gossen and Boujard, 1992). Expression of the p55-IC resulted in massive cell death (Fig. 3, right panel). The dying cells displayed cell surface blabbing as observed in the killing of the cells by TNF. Transfection of the p55-IC construct to the cells in the presence of tetracycline, which reportedly decreases the expression of pHD10-3 regulated constructs by as much as 10^5 fold, still resulted in some cell death, although significantly less than that observed in the absence of tetracycline (Fig 3, left panel). In contrast, cells transfected with a control construct, containing the lucipherase cDNA, showed no signs of death (results not shown).

The ability of the p55-IC to trigger cell death, when expressed without the transmembrane or extracellular domains of the receptor, provides further evidence for the involvement of this domain in signaling. Furthermore, it indicates that no other part of the receptor plays a direct role in such signaling.. Studies of the effects of mutations, including those mutations studied in the present invention, on the function of the p55-IC, indicated that the region extending between amino acid residues 326 and 407 is most critical for its

function. This region shows marked resemblance to sequences within the intracellular domains of two other receptors, evolutionarily related to the p55 TNF-R - namely, the Fas receptor (Itoh et al., 1991; Oehm et al., 1992), which can also signal for cell death and CD40 -a receptor (Stamenkovic et al., 1989) which enhances cell growth; this sequence therefore seems to constitute a conserved motif which plays some kind of general role in signaling. Since it does not resemble known motives characteristic of enzymatic activities, it seems plausible that it signals in indirect manner, i.e. possibly by serving as a docking site for signaling enzymes or for proteins which transmit stimulatory signals to them. The p55-IC, the Fas receptor and CD 40 can all be stimulated by antibodies against their extracellular domain. Their stimulation could be shown to correlate with the ability of the antibodies to cross-link the receptors. It therefore seems that the signaling is initiated as a consequence of interaction of two or more intracellular domains imposed by aggregation of the extracellular domains. Involvement of such interaction in the initiation of signaling of these receptors was also indicated by studies (Brakebusch et al., 1992) showing that expression of receptors made nonfunctional by mutation of their intracellular domain, had a "dominant negative" effect on the function of co-expressed normal receptors. Aggregation of the p55-R in response to TNF was suggested to occur in a passive manner, merely due to the fact that each of the TNF molecules, which occur as homotrimers, can bind two or three receptor molecules. However, the findings of the present invention suggest that this process occurs somewhat differently.

The propensity of the p55-IC to self associate indicates that this domain plays an active role in its induced aggregation. Moreover, this activity of the p55-IC seems to suffice for initiating its signaling, since when expressed independently of the rest of the receptor molecule, it can trigger cell death in the absence of TNF or any other exterior stimuli. Nevertheless, when expressed as the full length receptor, the p55-TNF-R does not signal, unless stimulated by TNF. One must, therefore, assume that when activating the p55-TNF-R, TNF actually overcomes some inhibitory mechanisms, which prevent spontaneous association of the intracellular domains, and this inhibition is due to the linkage of the p55-IC to the rest of the receptor molecule. The inhibition may be due to the

orientation imposed on the intracellular domain by the transmembrane and extracellular domain, to association of some other proteins with the receptor or perhaps just due to restriction of the amounts of receptors that are allowed to be placed in the plasma membrane. Of note, this control mechanism should be rather effective, since according to some estimations, the binding of even just one TNF molecule to a cell suffices for the triggering of its death.

Spontaneous signaling, independent of ligand can result in extensive derangement of the process controlled by this receptor. The best known example is the deregulation of growth factor receptors. Mutations due to which they start signaling spontaneously, for example those that cause them to aggregate spontaneously, play an important role in the deregulated growth of tumor cells. TNF effects, when induced in excess, are well known to contribute to the pathology of many diseases. The ability of free intracellular domains (p55ICs) of the p55-TNF-R to signal independently of TNF may contribute to such excessive function. It seems possible, for example, that some of the cytopathic effects of viruses and other pathogens result, not from their direct cytocidal function, but from proteolytic detachment of the intracellular domain of the p55-TNF-R and the resulting TNF-like cytotoxic effect.

In addition to the above-mentioned, the present inventors have also recently shown (see IL 111125) that a region within the p55IC, the so-called 'death domain' or p55DD, is primarily responsible for the self-association capability and hence the ligand-independent cell cytotoxicity. Further, the present inventors have also disclosed the self-association capability of the FAS-IC (see also IL 111125). Accordingly, these p55IC, p55DD and FAS-IC proteins and the DNA sequences encoding them are known and have been utilized for isolating and identifying additional IC-binding proteins (See Example 3 below).

As regards the cytotoxc activity of the intracellular domains of the p55 TNF-R and FAS-R (p55IC and FAS-IC) it has now also been further elucidated that both the p55IC, its 'death domain' (p55DD) and the FAS-IC are capable of a ligand-independent triggering of a cytocidal effect in HeLa cells. In this study, HeLa cells were transfected with expression vectors containing various constructs of either the full-length p55-TNF-R,

portions thereof including the p55IC and p55DD or the FAS-IC. In one set of experiments HeLa cells were co-transfected with constructs containing the p55 TNF-R (p55-R) and the FAS-IC (for details of the constructs, their preparation, etc. see above and IL 111125). The results of this study are depicted in Fig. 4 (A and B), wherein in both Fig. 4A and B the constructs used for transfecting the HeLa cells are shown schematically in the left hand panels; the results of the TNF or FAS receptor expression are shown graphically in the two middle panels (second and third panels from the left); and the results of transfected cell viability are shown graphically in the right hand panels. In Fig. 4A there is shown the results of transfected HeLa cells transiently expressing the full-length p55-R, p55-IC or parts thereof, or as a control, luciferase (LUC), in all cases using a tetracycline-controlled expression vector. In Fig. 4B there is shown the results of transfected HeLa cells transiently expressing FAS-IC alone or together with the p55-R, using a tetracycline-controlled expression vector. In the graphic representation of the results in Fig. 4A and B, the open bars represent cells transfected in the presence of tetracycline (1 μg/ml), which inhibits expression, and the closed bars represent cells transfected in the absence of tetracycline. TNF receptor expression was assessed 20h after transfection, both by ELISA using antibodies against the extracellular domain of the receptor (see left hand panels), and by determining the binding of radiolabeled TNF to the cells (middle panels). The cytocidal effect of the transfected proteins was assessed 48h after transfection. The data shown are from one of three experiments with qualitatively similar results in which each construct was tested in duplicate. The designation 'ND' in Figs. 4A and B means not determined. From the results shown in Figs. 4A and B it is apparent that expression of only the p55IC results in even greater cytotoxicity. Significant cytotoxicity also occurs when expressing just the death domain (p55DD). In contrast, expression of parts of p55IC lacking the death domain or containing only part thereof, had no effect on cell viability. Expression of the FAS-IC did not result in significant cytotoxicity, yet it significantly enhanced the cytotoxicity of co-expressed p55-R.

EXAMPLE 3 :

Additional proteins capable of binding to the intracellular domains of p55 TNF-R or FAS-R

Using the same approach and technology set forth in Example 1 above, four more proteins have been isolated and identified which are capable of binding to the p55IC or FAS-IC.

The first of these additional proteins called HF1, and now also called MORT-1 for "Mediator of Receptor-induced Toxicity", was identified, isolated and characterized as follows :

(i) **Two-hybrid screen and two-hybrid β-galactosidase expression test**

Two-hybrid screening (Fields and Song, 1989) of a Gal4 activation domain-tagged HeLa cell cDNA library (Clontech, Palo Alto, Ca., USA), using the intracellular domain of FAS-R (residues 175-319, Fas-IC) as 'bait', and β-galactosidase expression tests were performed as described above, except that the expression of β-galactosidase was assessed by a filter assay. In the screening, 5 of about $3x10^6$ cDNAs were found to contain the HF1 insert. Residue numbering in the proteins encoded by the cDNA inserts are as in the Swiss-Prot data bank. Deletion mutants were produced by PCR, and point mutants by oligonucleotide-directed mutagenesis (Current protocols in molecular biology, 1994).

(ii) **Induced expression, metabolic labeling and immunoprecipitation of proteins**

HF1, N-linked to the FLAG octopeptide (FLAG-HF1; Eastman Kodak, New Haven, Ct., USA), Fas-IC, FAS-R, p55-R, a chimera comprised of the extracellular domain of p55-R (amino acids 1-168) fused to the transmembrane and intracellular domain of FAS-R (amino acids 153-319), and the luciferase cDNA which serves as a control, were expressed in HeLa cells. Expression was carried out using a tetracycline-controlled expression vector, in a HeLa cell clone (HtTA-1) that expresses a tetracycline-controlled transactivator ((Gossen and Bujard, 1992), as described above (see also Boldin et al., 1995). Metabolic labeling with [^{35}S]methionine and [^{35}S]cysteine (DUPONT, Wilmington, De., USA and Amersham, Buckinghamshire, England) was performed 18

hours after transfection, by a further 4h incubation at 37°C in Dulbecco's modified Eagle's medium lacking methionine and cysteine, but supplemented with 2% dialyzed fetal calf serum. The cells were then lyzed in RIPA buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% NP-40, 1% deoxycholate, 0.1% SDS and 1 mM EDTA) and the lysate was precleared by incubation with irrelevant rabbit antiserum (3 µl/ml) and Protein G Sepharose beads (Pharmacia, Uppsala, Sweden; 60 µl/ml). Immunoprecipitation was performed by 1h incubation at 4°C of 0.3 ml aliquots of lysate with mouse monoclonal antibodies (5 µl/aliquot) against the FLAG octopeptide (M2; Eastman Kodak), p55-R (#18 and #20; (Engelmann et al., 1990)), or FAS-R (ZB4; Kamiya Southand Oaks, Ca., USA), or with isotype matched mouse antibodies as a control, followed by a further 1h incubation with Protein G Sepharose beads (30 µl/aliquot).

(iii) ***In vitro* binding**

Glutathione S-transferase (GST) fusions with the wild type or a mutated Fas-IC were produced and adsorbed to glutathione-agarose beads as described above (see also Boldin et al., 1995; Current protocols in molecular biology, 1994; Frangioni and Neel, 1993). Binding of metabolically-labeled FLAG-HF1 fusion protein to GST-Fas-IC was assessed by incubating the beads for 2h at 4°C with extracts of HeLa cells, metabolically labeled with [^{35}S]methionine (60 µCi/ml), that express FLAG-HF1. The extracts were prepared in a buffer containing 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% NP-40, 1 mM dithiotreitol, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 20 µg/ml Aprotonin, 20 µg/ml Leupeptin, 10 mM sodium fluoride and 0.1 mM sodium vanadate (1 ml per $5x10^5$ cells).

(iv) **Assessment of the cytotoxicity triggered by induced expression of HF1**

HF1, Fas-IC, p55-IC and luciferase cDNAs were inserted into a tetracycline-controlled expression vector and transfected to HtTA-1 cells (a HeLa cell line) (Gossen and Bujard, 1992) together with the secreted placental alkaline phosphatase cDNA, placed under control of SV40 promoter (the pSBC-2 vector (Dirks et al., 1993)). Cell death was assessed 40 hours after transfection, either by the neutral-red uptake assay (Wallach, 1984)

or, for assessing specifically the death in those cells that express the transfected cDNAs, by determining the amounts of placental alkaline phosphatase (Berger et al., 1988) secreted to the growth medium at the last 5 hours of incubation.

(v) **Northern and sequence analyses**

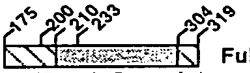
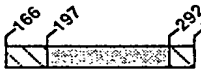
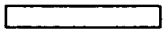
poly A^+ RNA was isolated from total RNA of HeLa cells (Oligotex-dT mRNA kit. QIAGEN, Hilden, Germany). Northern analysis using the HF1 cDNA as a probe was performed by conventional methods as described above (Example 1; see also Boldin et al., 1995). The nucleotide sequence of HF1 was determined in both directions by the dideoxy chain termination method.

The results (shown in Table 2 and Figs. 5-9) obtained from the above experimental procedures are as follows : Sequence analysis of the cDNA cloned by the two-hybrid procedure indicated that it encodes a novel protein (see below). Applying the two-hybrid test further to evaluate the specificity of the binding of this protein (called HF1 or MORT1 for "Mediator of Receptor-induced Toxicity") to Fas-IC, and to define the particular region in Fas-IC to which it binds, led to the following findings (Table 2) : (a) The protein binds both to human and to mouse Fas-IC, but not to several other tested proteins, including three receptors of the TNF/NGF receptor family (p55 and p75 TNF receptors and CD40); (b) Replacement mutations at position 225 (Ile) in the 'death domain' of FAS-R, shown to abolish signaling both *in vitro* and *in vivo* (the lpr^{cg} mutation (Watanabe-Fukunaga et al., 1992; Itoh and Nagata, 1993), also prevents binding of HF1 to the FAS-IC; (c) The HF1 binding-site in FAS-R occurs within the 'death domain' of this receptor; and (d) HF1 binds to itself. This self-binding, and the binding of HF1 to FAS-R involve different regions of the protein : A fragment of HF1 corresponding to residues 1-117 binds to the full-length HF1, but does not bind to itself nor to the FAS-IC. Conversely, a fragment corresponding to residues 130-245 binds to FAS-R, yet does not bind to HF1 (Table 2). Furthermore, it is apparent from the results in Table 2 that the 'death domain' region of FAS-R is critical for FAS-IC self-association, as is the 'death domain' region of p55-R for p55-IC self-association. The deletions on both sides of these 'death domains' does not affect the self-association ability thereof while, however, a deletion within these

'death domains' does affect the self-association. In the case of HF1, the binding of HF1 to FAS-IC is also dependent upon the complete (full) 'death domain' of FAS-R, while however, it is also not dependent on the regions outside of the FAS-R 'death domain' region for FAS-IC binding.

Table 2

Interaction of HF1 with FAS-IC and self-association of HF1 within transformed yeasts : assessment by a two-hybrid β-galactosidase expression test

DNA-BINDING - DOMAIN HYBRID	ACTIVATION-DOMAIN HYBRID: MORT1 Full	MORT1 1-117	MORT1 130-245	human Fas-IC	SNF4	pGAD-GH
Human Fas-IC						
 Full	++	-	+	++	-	-
'Death Domain'						
 200-319	++			++	-	-
 233-319	-			-	-	-
 175-304	++	-	+	++	-	-
Mouse Fas-IC						
 Full	++	-	++	++	-	-
 197-306	++	-	+	++	-	-
 I225N	-	-	-	++	-	-
 I225A	-			++	-	-
MORT1						
 Full	++	++	-	++	-	-
'Death Domain motif'						
 1-117	+	-		-	-	-
 130-245	-		-	++	-	-
SPECIFICITY TESTS						
human p55-IC	-			-	-	-
human p75-IC	-			-	-	-
human CD40-IC	-			-	-	-
Cyclin D	-			-	-	-
Lamin	-			-	-	-
SNF1	-			-	+	-
pGBT9	-			-	-	-

In Table 2 above there is depicted the interaction of the proteins encoded by the Gal4 DNA binding domain and activation-domain constructs (pGBT9 and pGAD-GH) within transfected SFY526 yeasts as assessed by β-galactosidase expression filter assay. The DNA-binding-domain constructs included four constructs of the human Fas-IC, four constructs of the mouse Fas-IC including two full-length constructs having Ile to Leu or Ile to Ala replacement mutations at position 225 (I225N and I225A, respectively), and three HF1 constructs, all of which constructs are shown schematically on the left-hand side of the table. The activation-domain constructs included three HF1 constructs, the HF1 portion being as in the DNA-binding-domain constructs; and a full-length human Fas-IC construct, the Fas-IC portion being the same as in the above DNA-binding domain construct. The intracellular domains of human p55 TNF receptor (p55-IC residues 206-426), human CD40 (CD40-IC, residues 216-277) and human p75 TNF receptor (p75-IC, residues 287-461) as well as lamin, cyclin D and 'empty' Gal4 (pGBT9) vectors served as negative controls in the form of DNA-binding domain constructs. SNF-1 and SNF4 served as positive controls in the form of DNA-binding-domain (SNF1) and activation-domain (SNF4) constructs. 'Empty" Gal4 vectors (pGAD-GH) also served as negative controls in the form of activation domain constructs. The symbols "++" and "+" denote the development of strong color within 30 and 90 min of the assay, respectively; and "-" denotes no development of color within 24h. Combinations for which no score is given have not been tested.

Expression of HF1 molecules fused at their N terminus with the FLAG octapeptide (FLAG-HF1) yielded in HeLa cells proteins of four distinct sizes - about 27, 28, 32, and 34 kD. In Fig. 5 (A and B) there is shown the results demonstrating the interaction of HF1 with Fas-IC *in vitro*. As noted above in the description of Figs. 5A and B, Fig. 5A is a reproduction of a control autoradiogram of an immunoprecipitate of proteins from extracts of HeLa cells transfected with the FLAG-HF1 (FLAG-MORT1) fusion protein or with luciferase cDNA as a control, the immunoprecipitation being performed with anti-FLAG antibody (αFLAG). Fig. 5B is a reproduction of an autoradiogram showing the interaction *in vitro* between HF1 and FAS-IC wherein the HF1 is in the form of [^{35}S]methionine-

metabolically labeled HF1-FLAG fusion proteins obtained from extracted of transfected HeLa cells and the FAS-IC is in the form of human and mouse GST-FAS-IC fusion proteins including one having a replacement mutation at position 225 in FAS-IC, all of which GST-FAS-IC fusion proteins were produced in *E. coli*. The GST-fusion proteins were attached to glutathione beads before interaction with the extracts containing the HF1-FLAG fusion protein following this interaction, SDS-PAGE was performed. Thus the *in vitro* interaction was evaluated by assessing, by autoradiography following SDS-PAGE, the binding of [^{35}S] metabolically labeled HF1, produced in transfected HeLa cells as a fusion with the FLAG octapeptide (FLAG-HF1), to GST, GST fusion with the human or mouse Fas-IC (GST-huFas-IC, GST-mFas-IC) or to GST fusion with Fas-IC containing a Ile to Ala replacement mutation at position 225. As is apparent from Fig. 5B, all four FLAG-HF1 proteins showed ability to bind to Fas-IC upon incubation with a GST-Fas-IC fusion protein. As in the yeast two-hybrid test (Table 2), HF1 did not bind to a GST-Fas-IC fusion protein with a replacement at the *lpr*^{cg} mutation site (I225A).

The proteins encoded by the FLAG-HF1 cDNA showed also an ability to bind to the intracellular domain of FAS-R, as well as to the intracellular domain of FAS-R chimera whose extracellular domain was replaced with that of p55-R (p55-FAS), when co-expressed with these receptors in HeLa cells. In Fig. 6 (A, B, C) there is shown the results demonstrating the interaction of HF1 with FAS-IC in transfected HeLa cells, i.e. *in vivo*. As mentioned above in the description of Figs. 6 A, B, C, these figures are reproductions of autoradiograms of immunoprecipitates of various transfected HeLa cells which demonstrate the *in vivo* interaction and specificity of the interaction between HF1 and FAS-IC in cells co-transfected with constructs encoding these proteins. Thus, FLAG-HF1 fusion protein was expressed and metabolically labeled with [^{35}S] cystein (20 μCi/ml) and [^{35}S]methionine (40 μCi/ml) in HeLa cells, alone, or together with human FAS-R, FAS-R chimera in which the extracellular domain of FAS-R was replaced with the corresponding region in the human p55-R (p55-FAS), or the human p55-R, as negative control. Cross immunoprecipitation of HF1 with the co-expressed receptor was performed using the indicated antibodies (Figs. 6 A-C). As is apparent in Figs. 6 A-C, FLAG-HF1 is capable of

binding to the intracellular domain of FAS-R, as well as to the intracellular domain of a FAS-R-p55-R chimera having the extracellular domain of p55-R and the intracellular domain of FAS-R, when co-expressed with these receptors in the HeLa cells (see 3 middle lanes Fig. 6A and 3 left-hand lanes Fig. 6C, respectively). Further, immunoprecipitation of FLAG-HF1 from extracts of the transfected cells also resulted in precipitation of the co-expressed FAS-R (Fig. 6A) or the co-expressed p55-FAS chimera (Fig. 6C). Conversely, immunoprecipitation of these receptors resulted in the coprecipitation of the FLAG-HF1 (Figs. 6A and 6C).

Northern analysis using the HF1 cDNA as probe revealed a single hybridizing transcript in HeLa cells. In Fig. 7 there is shown a reproduction of a Northern blot in which poly A^+ RNA (0.3 μg) from transfected cells was hybridized with the HF1 cDNA. The size of this transcript (about 1.8 kB) is close to that of the HF1 cDNA (about 1702 nucleotides).

In sequence analysis, the cDNA was found to contain an open reading frame of about 250 amino acids. In Fig. 8 there is depicted the preliminary nucleotide and deduced amino acid sequence of HF1 in which the 'death domain' motif is underlined, as is a possible start Met residue (position 49; bold, underlined M) and the translation stop codon (the asterisk under the codon at position 769-771). This 'death domain' motif shares homology with the known p55-R and FAS-R 'death domain' motifs (p55DD and FAS-DD). In order to determine the precise C-terminal end of HF1 and to obtain evidence concerning the precise N-terminal (initial Met residue) end of HF1, additional experiments were carried out as follows :

Using the methods described above, a number of constructs encoding HF1 molecules fused at their N-terminus with the FLAG octapeptide (FLAG-HF1) were constructed and expressed in HeLa cells with metabolic labeling of the expressed proteins using ^{35}S-cysteine and ^{35}S-methionine (see the above in respect of Fig. 5B). The HF1-FLAG molecules were encoded by the following cDNAs containing different portions of the HF1-encoding sequence :

i) The FLAG octapeptide cDNA linked to the 5' end of the HF1 cDNA from which nucleotides 1-145 (see Fig. 8) have been deleted;

ii) The FLAG octapeptide cDNA linked to the 5' end of the HF1 full length cDNA (see FLAG-HF1 construct above in respect of Fig. 5B);

iii) The FLAG octapeptide cDNA linked to the 5' end of the HF1 cDNA from which nucleotides 1-145 as well as nucleotides 832-1701 (see Fig. 8) have been deleted and the codon GCC at position 142-144 was mutated to TCC to prevent start of translation at this site.

Following expression of the above HF1-FLAG fusion products, immunoprecipitation was carried out as mentioned above, using either anti-FLAG monoclonal antibodies (M2) or as a control, anti-p75 TNF-R antibodies (#9), followed by SDS-PAGE (10% acrylamide) and autoradiography. The results are shown in Fig. 9 which is a reproduction of an autoradiogram on which was separated the above noted HF1-FLAG fusion proteins, the samples loaded on each lane of the gel being as follows :

Lanes 1 and 2 - HF1-FLAG fusion protein encoded by the FLAG octapeptide cDNA linked to the 5' end of the HF1 cDNA from which nucleotides 1-145 were deleted

Lanes 3 and 4 - HF1-FLAG fusion protein encoded by the FLAG octapeptide cDNA linked to the 5' end of the full length HF1 cDNA

Lanes 5 and 6 - HF1-FLAG fusion protein encoded by the FLAG octapeptide cDNA linked to the 5' end of the HF1 cDNA from which nucleotides 1-145 as well as 832-1701 have been deleted and the GCC at position 142-144 was mutated to TCC to prevent start of translation at this site.

The immunoprecipitations were with anti-FLAG monoclonal antibodies for the samples in lanes 2, 4 and 6 and anti-p75 TNF-R antibodies for the samples in lanes 1, 3 and 5.

From the autoradiogram of Fig. 9 it is apparent that the identity of product sizes in lanes 2 and 4 confirms that the nucleotides 769-771 are the site of translation termination for HF1, i.e. this codon represents a stop signal and is indicated by an asterisk in Fig. 8. Further, the occurrence of a broad band which represents just two translation products (as seen on the gel but being strongly labeled becomes a single large band on the

autoradiogram) in lane 6 indicates that the occurrence of two additional products (the higher molecular weight broad bands) in lanes 2 and 4 reflect the initiation of translation both at the N-terminus of the FLAG-HF1 fusion molecule and at the methionine residue number 49 within the HF1 sequence (see bold underlined M at position 49 of the amino acid sequence in Fig. 8). Thus, the above results have confirmed (validated) the C-terminal end of HF1 and have provided evidence that the N-terminal end of HF1 may be at position 49 of the sequence in Fig. 8 (the precise N-terminal end still to be determined).

It should be mentioned that a search conducted in the 'Gene Bank' and 'Protein Bank' DataBases revealed that there is no sequence corresponding to that of HF1 depicted in Fig. 8. Thus, HF1 represents a new FAS-IC-specific binding protein.

High expression of p55-IC results in triggering of a cytocidal effect (see Example 2 above and Boldin et al., 1995). The expression of Fas-IC in HeLa cells also has such an effect, though to a lower extent, which could be detected only with the use of a sensitive assay. In Fig. 10 A and B there is depicted graphically the ligand independent triggering of cytocidal effects in cells transfected with HF1, as well as human p55-IC and FAS-IC. The effect of transient expression of HF1, human Fas-IC, human p55-IC, or luciferase that served as a control, on the viability of HeLa cells was assessed using a tetracycline-controlled expression vector. Cell viability was evaluated 40 min after transfecting these cDNAs either in presence (open bars, Figs. 10A and B) or absence (closed bars, Fig. 10A and B) of tetracycline (1 μg/ml, to block expression), together with a cDNA encoding the secreted placental alkaline phosphatase. Cell viability was determined either by the neutral red uptake assay (Fig. 10A) or, for determining specifically the viability of those particular cells that express the transfected DNA, by measuring the amounts of placental alkaline phosphatase secreted to the growth medium (Fig. 10B).

Thus, it is apparent from Figs. 10 A and B that the expression of HF1 in HeLa cells resulted in significant cell death, greater than that caused by FAS-IC expression. These cytotoxic effects of all of p55-IC, FAS-IC and HF1 seem to be related to the 'death domain' regions, present in all of these proteins, which 'death domains' have a propensity to self-associate, and thereby possibly prompting the cytotoxic effects.

In view of the above mentioned characteristics of HF1 (MORT-1), namely, the specific association of HF1 with that particular region in FAS-R which is involved in cell death induction, and the fact that even a slight change of structure in that region, which prevents signaling (the *lpr*^{cg} mutation) abolishes also the binding of HF1, indicates that this protein plays a role in the signaling or triggering of cell death. This notion is further supported by the observed ability of HF1 to trigger by itself a cytocidal effect. Thus, HF1 (MORT-1) may function as (i) a modulator of the self-association of FAS-R by its own ability to bind to FAS-R as well as to itself, or (ii) serve as a docking site for additional proteins that are involved in the FAS-R signaling, i.e. HF1 is probably a 'docking' protein and may therefore bind other receptors besides FAS-R, or (iii) constitutes part of a distinct signaling system that interacts with FAS-R signaling.

Moreover, HF1 may be utilized to specifically identify, isolate and characterize other proteins which are capable of binding to HF1 (i.e. HF1-binding proteins), and which may be utilized directly to modulate or mediate the above HF1-associated effects on cells, or, indirectly, to mediate or modulate the FAS-R ligand-effect on cells when this effect is modulated or mediated by HF1.

Using the same approach as noted above in respect of HF1, the other three additional proteins capable of binding to the intracellular domains of p55 TNF-R or FAS-R were identified, isolated and characterized (partially).

In Figs. 11-13 there is shown schematically the partial and preliminary nucleotide sequence of cDNA clones, called F2, F9 and DD11, respectively.

Clones F2 and F9 were isolated by screening a murine (mouse) embryonic library using the murine FAS-IC as "bait". In Fig. 11 there is shown schematically the partial nucleotide sequence from the F2 cDNA that has been sequenced. In Fig. 12 there is shown schematically the partial nucleotide sequence of 1724 bases from the F9 cDNA that has been sequenced. Analysis of the binding capability of the protein encoded by clones F2 and F9 (F2 and F9, respectively) has shown that :

(a) F2 interacts strongly with human p55IC and p55DD and with murine FAS-IC, while it interacts weakly with non-relevant (control) proteins SNF1 and Lamin as well as relevant protein, human FAS-IC.

(b) F9 interacts strongly with human p55-IC and murine FAS-IC, while it interacts weakly with human FAS-IC (relevant protein) and irrelevant proteins SNF1 and Lamin.

(c) Neither F2 nor F9 interacted at all with human p75IC, pGBT9 (empty bait vector), or human CD-40.

Further, from 'Gene Bank' and 'Protein Bank' searches it was revealed that F2 and F9 represent new proteins.

Thus, F2 and F9 represent new proteins having binding specificity for both FAS-IC and p55IC.

Clone DD11 was isolated by screening a human HeLa library using the human p55DD as "bait". In Fig. 13 there is shown schematically the partial nucleotide sequence of 425 bases from the DD11 cDNA that has been sequenced.

The DD11 clone has an approx. length of 800 nucleotides. The full length of the transcript is about 1.2 kb, the transcript having been probed using the clone. Analysis of the binding capability of the protein encoded by clone DD11 has shown that DD11 interacts strongly with the p55DD (a.a. 326-414) (see Fig. 4) and does not interact with deletion mutants of this domain, e.g. a.a. 326-404. DD11 also interacts with mouse and human FAS-IC and to some extent also with Lamin. DD11 does not interact at all with SNF1 nor with pGBT9 (empty bait vector). DD11 is also not found in the 'Gene Bank' and 'Protein Bank' databases. Thus DD11 represents a p55 IC (p55DD) and FAS-IC specific binding protein.

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CLAIMS

1. A DNA sequence encoding a protein capable of binding to one or more of the intracellular domains of one or more receptors belonging to the tumor necrosis factor/nerve growth factor (TNF/NGF) receptor superfamily.

2. A DNA sequence according to claim 1, wherein said receptors are the TNF-Rs, p55 TNF-R or p75 TNF-R, or the FAS ligand receptor (FAS-R).

3. A DNA sequence according to claim 1 or claim 2, selected from the group consisting of:

   (a) a cDNA sequence derived from the coding region of a native TNF-R or FAS-R intracellular domain-binding protein;

   (b) DNA sequences capable of hybridization to a sequence of (a) under moderately stringent conditions and which encode a biologically active TNF-R or FAS-R intracellular domain-binding protein; and

   (c) DNA sequences which are degenerate as a result of the genetic code to the DNA sequences defined in (a) and (b) and which encode a biologically active TNF-R or FAS-R intracellular domain-binding protein.

4. A DNA sequence according to any one of claims 1-3, encoding a p55 TNF-R intracellular domain (p55IC)-binding protein.

5. A DNA sequence according to claim 4 encoding a protein selected from the group comprising the herein designated proteins 55.1, 55.3, 55.11, F2, F9 and DD11.

6. A DNA sequence according to claim 5, selected from the sequences contained in the herein designated cDNA clones 55.1, 55.3, 55.11, F2, F9 and DD11.

7. A DNA sequence according to any one of claims 1-3, encoding a p75 TNF-R intracellular domain (p75IC)-binding protein.

8. A DNA sequence according to claim 7, encoding a protein selected from the group comprising the herein designated proteins 75.3 and 75.16.

9. A DNA sequence according to claim 8, selected from the sequences contained in the herein designated cDNA clones 75.3 and 75.16.

10. A DNA sequence according to claim 5 or claim 6 encoding the protein 55.1 having the amino acid sequence from amino acid residue 328 to residue 426 of the p55 TNF-R amino acid sequence.

11. A DNA sequence according to claim 5 of claim 6 encoding the protein 55.3 having the amino acid sequence from amino acid residue 277 to residue 426 of the p55 TNF-R amino acid sequence.

12. A DNA sequence according to claim 5 or claim 6, encoding the protein 55.11 comprising the sequence depicted in Fig. 1(a).

13. A DNA sequence according to claim 8 or claim 9 encoding the protein 75.3 comprising the sequence depicted in Fig. 1(b).

14. A DNA sequence according to claim 8 or claim 9 encoding the protein 75.16 comprising the sequence depicted in Fig. 1(c).

15. A DNA sequence according to any one of claims 1-3 encoding a FAS-R intracellular domain (FAS-IC)-binding protein.

16. A DNA sequence according to claim 15 encoding a protein selected from the group comprising the herein designated proteins HF1, F2, F9 and DD11.

17. A DNA sequence according to claim 16 selected from the sequences contained in the herein designated cDNA clones HF1, F2, F9 and DD11.

18. A DNA sequence according to claim 16 or 17 encoding any one of the proteins HF1, F2, F9 and DD11, comprising the sequences depicted in any one of Figs. 8, and 11-13, respectively.

19. A DNA sequence according to claim 18 encoding the protein HF1 (MORT-1), comprising the sequence depicted in Fig. 8.

20. A protein or analogs and derivatives thereof encoded by a sequence according to any one of claims 1-19, said protein, analogs and derivatives being capable of binding to one or more of the intracellular domains of one or more TNF-Rs or FAS-R.

21. A protein according to claim 20 selected from the group comprising the proteins 55.1, 55.3. 55.11, 75.3, 75.16, HF1, F2, F9 and DD11, and biologically active analogs and derivatives thereof.

22. The protein HF1 (MORT-1) according to claim 21, having the deduced amino acid sequence depicted in Fig. 8.

23. A vector comprising a DNA sequence according to any one of claims 1-19.

24. A vector according to claim 23 which is capable of being expressed in a eukaryotic host cell.

25. A vector according to claim 23 which is capable of being expressed in a prokaryotic host cell.

26. Transformed eukaryotic or prokaryotic host cells containing a vector according to any one of claims 23-25.

27. A method for producing the protein analogs or derivatives according to claim 20 or 21 comprising growing the transformed host cells according to claim 26 under conditions suitable for the expression of said protein, analogs or derivatives, effecting post-translational modifications of said protein as necessary for obtention of said protein and extracting said expressed protein, analogs or derivatives from the culture medium of said transformed cells or from cell extracts of said transformed cells.

28. Antibodies or active fragments or derivatives thereof, specific for the protein, analogs or derivatives according to claim 20 or claim 21.

29. A method for the modulation of the TNF or FAS-R ligand effect on cells carrying a TNF-R or a FAS-R, comprising treating said cells with one or more proteins, analogs or derivatives selected from the group consisting of the proteins, analogs and derivatives according to claim 20 or claim 21 and a protein being the p55IC, p55DD, FAS-IC or FAS-DD, analogs or derivatives thereof, all of said proteins being capable of binding to the intracellular domain and modulating the activity of said TNF-R or FAS-R, wherein said treating of said cells comprises introducing into said cells said one or more proteins, analogs or derivatives in a form suitable for intracellular introduction thereof, or introducing into said cells a DNA sequence encoding said one or more proteins, analogs or derivatives in the form of a suitable vector carrying said sequence, said vector being capable of effecting the insertion of said sequence into said cells in a way that said sequence is expressed in said cells.

30. A method for the modulation of the FAS-R ligand-effect on cells according to claim 29, comprising treating said cells with HF1 (MORT-1), analogs or derivatives thereof, all being capable of binding to the intracellular domain and modifying the activity of FAS-R, wherein said treating of cells comprising introducing into said cells said HF1, analogs or derivatives in a form suitable for intracellular introduction thereof, or introducing into said cells a DNA sequence encoding said HF1, analogs or derivatives in the form of a suitable vector carrying said sequence, said vector being capable of effecting the insertion of said sequence into said cells in a way that said sequence is expressed in said cells.

31. A method according to claim 29 wherein said treating of said cells is by transfection of said cells with a recombinant animal virus vector comprising the steps of :

(a) constructing a recombinant animal virus vector carrying a sequence encoding a viral surface protein (ligand) that is capable of binding to a specific cell surface receptor on the surface of a TNF-R- or FAS-R- carrying cell and a second sequence encoding a protein selected from the proteins, analogs and derivatives according to claim 20 or 21 and a protein being the p55IC, p55DD, FAS-IC or FAS-DD, analogs

or derivatives thereof, said protein when expressed in said cells being capable of modulating the activity of said TNF-R or FAS-R; and

(b) infecting said cells with said vector of (a).

32. A method according to claim 30 or claim 31, wherein said treating of cells is by transfection of said cells with a recombinant animal virus vector comprising the steps of:

(a) constructing a recombinant animal virus vector carrying a sequence encoding a viral surface protein (ligand) that is capable of binding to a specific cell surface receptor on the surface of a FAS-R-carrying cell and a second sequence encoding HF1, analogs and derivatives thereof, said HF1, analogs or derivatives, when expressed in said cells being capable of modulating the activity of said FAS-R; and

(b) infecting said cells with said vector of (a).

33. A method for modulating the TNF or FAS-R ligand effect on cells carrying a TNF-R or a FAS-R comprising treating said cells with antibodies or active fragments or derivatives thereof, according to claim 28, said treating being by application of a suitable composition containing said antibodies, active fragments or derivatives thereof to said cells, wherein when the IC-binding proteins of said cells are exposed on the extracellular surface, said composition is formulated for extracellular application, and when said IC-binding proteins are intracellular said composition is formulated for intracellular application.

34. A method for modulating the TNF or FAS-R ligand effect on cells carrying a TNF-R or FAS-R comprising treating said cells with an oligonucleotide sequence selected from a sequence encoding an antisense sequence of at least part of the sequence according to any one of claims 1-19, and a sequence encoding the antisense sequence of p55IC, p55DD, FAS-IC or FAS-DD, said oligonucleotide sequence being capable of blocking the expression of at least one of the TNF-R or FAS-R intracellular domain binding proteins.

35. A method according to claim 34 for modulating the FAS-R ligand-effect on cells carrying a FAS-R comprising treating said cells with an oligonucleotide sequence selected from a sequence encoding an antisense sequence of at least part of the HF1 sequence set forth in Figure 8, said oligonucleotide sequence being capable of blocking the expression of HF1.

36. A method according to claim 34 or 35 wherein said oligonucleotide sequence is introduced to said cells via a virus of claim 31 or 32 wherein said second sequence of said virus encodes said oligonucleotide sequence.

37. A method for treating tumor cells or HIV-infected cells or other diseased cells, comprising:

(a) constructing a recombinant animal virus vector carrying a sequence encoding a viral surface protein that is capable of binding to a specific tumor cell surface receptor or HIV-infected cell surface receptor or receptor carried by other diseased cells and a sequence encoding a protein selected from the proteins, analogs and derivatives of claims 20 and 21 and the p55 TNF-R intracellular domain (p55IC), its 'death domain' (p55DD), the intracellular domain of FAS-R (FAS-IC), or its 'death domain' (FAS-DD), or a biologically active analog or derivative thereof, said protein, when expressed in said tumor, HIV-infected, or other diseased cell being capable of killing said cell; and

(b) infecting said tumor or HIV-infected cells or other diseased cells with said vector of (a).

38. A method according to claim 37 for treating tumor cells, HIV-infected cells or other diseased cells, comprising :

(a) constructing a recombinant animal virus vector carrying a sequence encoding a viral surface protein that is capable of binding to a specific tumor cell surface receptor or HF1-infected cell surface receptor or another receptor present on other diseased cells and a sequence encoding HF1, analogs and derivatives thereof, said

HF1, analogs or derivatives thereof, when expressed in said tumor, HIV-infected or other diseased cell being capable of killing said cell; and

(b) infecting said tumor or HIV-infected or other diseased cells with said vector of (a).

39. A method for modulating the TNF or FAS-R ligand effect on cells comprising applying the ribozyme procedure in which a vector encoding a ribozyme sequence capable of interacting with a cellular mRNA sequence encoding a protein according to claim 20 or 21 or a mRNA sequence encoding p55IC, p55DD, FAS-IC or FAS-DD, is introduced into said cells in a form that permits expression of said ribozyme sequence in said cells, and wherein when said ribozyme sequence is expressed in said cells it interacts with said cellular mRNA sequence and cleaves said mRNA sequence resulting in the inhibition of expression of said protein or said p55IC, p55DD, FAS-IC or FAS-DD in said cells.

40. A method according to claim 39 for modulating the FAS-R ligand-effect on cells comprising applying the ribozyme procedure in which a vector encoding a ribozyme sequence capable of interacting with a cellular mRNA sequence encoding HF1 is introduced into said cells in a form that permits expression of said ribozyme sequence in said cells, and wherein when said ribozyme sequence is expressed in said cells it interacts with said cellular mRNA sequence encoding HF1 and cleaves said mRNA sequence resulting in inhibition of expression of HF1.

41. A method selected from the method according to any one of claims 30, 32, 35, 36, 38 and 40, wherein said HF1 protein or said HF1 encoding sequence comprises at least that part of the HF1 protein, which binds specifically to the FAS-IC, or at least that part of the HF1 encoding sequence that encodes that part of the HF1 protein which binds specifically to the FAS-IC.

42. A method for isolating and identifying proteins, factors or receptors capable of binding to the intracellular domain binding proteins according to claim 20 or 21,

comprising applying the procedure of affinity chromatography in which said protein according to claim 20 or 21 is attached to the affinity chromatography matrix, said attached protein is brought into contact with a cell extract and proteins, factors or receptors from cell extract which bound to said attached protein are then eluted, isolated analyzed.

43. A method for isolating and identifying proteins, capable of binding to the intracellular domain binding proteins according to claim 20 or 21, comprising applying the yeast two-hybrid procedure in which a sequence encoding said intracellular domain binding protein is carried by one hybrid vector and sequence from a cDNA or genomic DNA library are carried by the second hybrid vector, the vectors then being used to transform yeast host cells and the positive transformed cells being isolated, followed by extraction of the said second hybrid vector to obtain a sequence encoding a protein which binds to said intracellular domain binding protein.

44. A pharmaceutical composition for the modulation of the TNF- or FAS-R ligand-effect on cells comprising, as active, ingredient a protein according to claim 20 or 21, or the protein p55IC, p55DD, FAS-IC or FAS-DD, its biologically active fragments, analogs, derivatives or mixtures thereof.

45. A pharmaceutical composition for modulating the TNF- or FAS-R ligand- effect on cells comprising, as active ingredient, a recombinant animal virus vector encoding a protein capable of binding a cell surface receptor and encoding a protein according to claim 20 or 21, or the protein p55IC, p55DD, FAS-IC or FAS-DD, its biologically active fragments or analogs.

46. A pharmaceutical composition for modulating the TNF or FAS-R ligand effect on cells comprising as active ingredient, an oligonucleotide sequence encoding an anti-sense sequence of the sequence according to any one of claims 1-19.

47. A method for isolating and identifying a protein capable of binding to the intracellular domains of TNF-Rs or FAS-R comprising applying the procedure of non-stringent

southern hybridization followed by PCR cloning, in which a sequence or parts thereof according to any one of claims 1-19 is used as a probe to bind sequences from a cDNA or genomic DNA library, having at least partial homology thereto, said bound sequences then amplified and cloned by the PCR procedure to yield clones encoding proteins having at least partial; homology to said sequences of claims 1-19.

48. A method for the modulation of the HF1-induced effect on cells comprising treating said cells in accordance with a method according to any one of claims 29-41 with HF1, analogs or derivatives thereof or with sequences encoding HF1 or analogs thereof, said treatment resulting in the enhancement or inhibition of said HF1-mediated effect.

49. A method according to claim 48 wherein said HF1 protein, analog of derivative thereof is that part of HF1 which is specifically involved in binding to HF1 itself, or said HF1 sequence encodes that part of HF1 which is specifically involved in binding to HF1 itself.

50. A DNA sequence encoding a protein capable of binding to HF1 (MORT-1).

51. A DNA sequence according to claim 50 selected from the group consisting of :

(a) a cDNA sequence derived from the coding region of a native HF1-binding protein;

(b) DNA sequences capable of hybridization to a sequence of (a) under moderately stringent conditions and which encode a biologically active HF1-binding protein; and

(c) DNA sequences which are degenerate as a result of the genetic code to the DNA sequences defined in (a) and (b) and which encode a biologically active HF1-binding protein.

52. An HF1-binding protein or analogs and derivatives thereof encoded by a sequence according to claim 50 or claim 51.

53. A vector comprising a DNA sequence according to claim 50 or 51, which is capable of being expressed in a prokaryotic or eukaryotic host cell.

54. Transformed eukaryotic or prokaryotic host cells containing a vector according to any one of claims 23-25.

55. A method for producing the HF1-binding protein, analogs or derivatives thereof according to claim 52, comprising growing the transformed host cells according to claim 54 under conditions suitable for the expression of said protein, analogs or derivatives, effecting post-translational modifications of said protein as necessary for obtention of said protein and extracting said expressed protein, analogs or derivatives from the culture medium from said transformed cells or from cell extracts of said transformed cells.

56. Antibodies or active fragments or derivatives thereof, specific for the HF1-binding protein, analogs or derivatives according to claim 52.

57. A method for the modulation of the HF1-associated effect on cells according to claim 48, said method being selected from the methods of any one of claims 29-41, wherein the HF1-binding protein, analogs or derivatives thereof or sequences encoding the HF1-binding protein, or analogs thereof are introduced into the cells and result in the inhibition or enhancement of the HF1-associated effect.

58. A method for the isolation and identification of HF1-binding proteins, said method being selected from the methods according to claim 42 or claim 43, wherein the HF1 protein is utilized by the procedure of affinity chromatography to isolate HF1-binding proteins from a biological sample; or wherein the HF1 encoding sequence is utilized in the yeast two-hybrid procedure to isolate from a cDNA or genomic DNA library a DNA sequence encoding the HF1-binding protein sequence.

For the Applicant

Henry Einav

55.11 clone

FIGURE 1(a)

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(b) 75.3

5'GAATTCGGCACGAGCGGCACGAGGACAGAGTGAGACTCTGTCTCTTAAAATAATAATA
AAAATAAAAATAAAATGTGGGGCCGGGCAAGGTGGCTCATGCCTGTAATCCCAGCACCTT
GGGAGGCTGAGGCAGGAGGATTGCCTAAGCCCAGGAGTTTGACATCAGCCTGGGCAACAT
GGTGAAACCCCATCTCTACAAAAAATGCAAAAATTAGCCAGGTGTGGTGGGTGTGCTCCT
ATAGTCTCAGCTACTCAGGAGGCTGAGGTAGAGGGGATCACCTGAGCCCAGGAAGTTTGG
AGGCTATAGTGAGCTGAAGACCCGCACCATTGCACGCCAGCCTGGAGCAAGAGACNCTGT
CTCCACATAAATAAATAAATAAATAAAAGTGGGGAACTTCTGTGTTAAGTCAGAAGGCAC
CACACAATTTGNATAGCCANCAACCATATTCAATACCCAATCTCTTTATTGCAATATAAG
TATTTGTAAACCCCTACACAAATATTCCCAAGAATAAGTTGGAATATAAATTACTATATC
AATCANCCAATAAAAATAAACACATACAGTATTTATTTCCTGTTGCTCCATATAAAGCTT
TGCTATTTCAATATAAAGCTTACCTAGTATGGTCATTTGAGCCTGAGCAGAGAATATGCC
CAAGCTCGTGCCGAATTC....GGCGNTCTGACTCTCTACTGAACCAAGACTGAATCAGA
GAGACTCGAGTGCNCTTATTTGATTAANCCCAAATTATTGAAACCTNTGATTTTTTCTGG
AGGNGGATGATAAAGATGTGAAAGTGTGATGAACAGTGTGTATCCCTACTCTTGATCCTG
GAACCAGACAAGCAAGAAGCTTTGATTGAAAGCCTATGTGAAAAGCTGGTCAAATTTCGC
GAAGGTGAACGCCCGTCTCTGAGACTGCAGTTGTTAAGCAACCTTTTCCACGGGATGGAT
AAGAATACTCCTGTAAGATACACAGTGTATTGCAGCCTTATTAAAGTGGCAGCATCTTGT
GGGGCCATCCAGTACATCCCAACTGAGCTGGATCAAGTTAGAAAATGGATTTCTGACTGG
AATCTCACCACTGAAAAAAAGCACACCCTTTTAAGACTACTTTATGAGGCACTTGTGGAT
TGTAAGAAGAGTGATGCTGCTTCAAAAGTCATGGTGGAATTGCTCGGAAGTTACACAGAG
GACAATGCTTCCCAGGCTCGAGTTGATGCCCACAGGTGTATTGTACGAGCATTGAAAGAT
CCAAATGCATTTCTTTGTGACCACCTTCTTACTTTAAAACCAGTCAAGTTTGTGGAAGGC
GAGCTTATTCATGATCTTTTAACCATTTGTGTGAGTGCTAAATTGGCATCATATGTCAAG
TTTTATCAGAATAATAAAGACTTCATTGATTCACTTGGCCTGTTACATGAACAGAATATG
GCAAAAATGAGACTACTTACTTTTATGGGAATGGCAGTAGAAAATAAGGAAATTTCTTTT
GACACAATGCAGCAAGAACTTCAGATTGGAGCTGATGATGTTGAAGCATTTGTTATTGAC
GCCGTAAGAACTAAAATGGTCTACTGCAAAATTGATCAGACCCAGAGAAAAGTAGTTGTC
AGTCATAGCACACATCGGACATTTGGAAAACAGCAGTGGCAACAACTGTATGACACACTT
AATGCCTGGAAACAAAATCTGAACAAAGTGAAAAACAGCCTTTTGAGTCTTTCTGATACC
TGAGTTTTTATGCTTATAATTTTTGTTCTTTGAAAAAAAAGCCCTAAATCATAGTAAAAC
ATTATAAACTAAAAAAAAAAAAAAAAAACTCGAG 3'

FIGURE 1(b)

Yeda Research and Development Co. Ltd.

(c) 75.16

5'GTCCGGTTTACTTTAACTTAGTTTTGCATAGTTCTAGTGCACGTGAAATTGAAAAGTTA
TTTCCCTTTAGCTGTGTTATTATAGAGCAGAAATTCTGTTTTTAAAAATTAGCCTAAGATA
TACTTGTTTTTGTAAAGAAAAATATTTAATGCTTGAACAAAATAAATTGGAGTTGGAGTAG
AATGTAGTTTGAGGAAATTTGCAGCTTCCAATGCCTCTG.....CAGAGGCATTGGAAGCT
GCAAATTTCCTCAAACTACATTCTACTCCAACTCCAATTTATTTTGTTCAAGCATTAAATA
TTTTTCTTTACAAAAACAAGTATATCTTAGGCTAATTTTTAAAAACAGAATTTCTGCTCTA
TAATAACACAGCTAAAGGGAAATAACTTTTCAATTTCACGTGCACTAGAACTATGCAAAAC
TAAGTTAAAGTAAACCGGAC 3'

FIGURE 1(c)

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```
1/1                                                                 31/11
GTG AAT CAG GCA CCG GAG TGC AGG TTC GGG GGT GGA ATC CTT GGG CCG CTG GGC AAG CGG
V   N   Q   A   P   E   C   R   F   G   G   G   I   L   G   P   L   G   K   R
61/21                                                               91/31
CGA GAC CTG GCC AGG GCC AGC GAG CCG AGG ACA GAG GGC GCG CGG AGG GCC GGG CCG CAG
R   D   L   A   R   A   S   E   P   R   T   E   G   A   R   R   A   G   P   Q
121/41                                                              151/51
CCC CGG CCG CTT GCA GAC CCC GCC ATG GAC CCG TTC CTG GTG CTG CTG CAC TCG GTG TCG
P   R   P   L   A   D   P   A   M   D   P   F   L   V   L   L   H   S   V   S
181/61                                                              211/71
TCC AGC CTG TCG AGC AGC GAG CTG ACC GAG CTC AAG TTC CTA TGC CTC GGG CGC GTG GTC
S   S   L   S   S   S   E   L   T   E   L   K   F   L   C   L   G   R   V   V
241/81                                                              271/91
AAG CGC AAG CTG GAG CGC GTG CAG AGC GGC CTA GAC CTC TTC TCC ATG CTG CTG GAG CAG
K   R   K   L   E   R   V   Q   S   G   L   D   L   F   S   M   L   L   E   Q
301/101                                                             331/111
AAC GAC CTG GAG CCC GGG CAC ACC GAG CTC CTG CGC GAG CTG CTC GCC TCC CTG CGG CGC
N   D   L   E   P   G   H   T   E   L   L   R   E   L   L   A   S   L   R   R
361/121                                                             391/131
CAC GAC CTG CTG CGG CGC GTC GAC GAC TTC GAG GCG GGG GCG GCG GCC GGG GCC GCG CCT
H   D   L   L   R   R   V   D   D   F   E   A   G   A   A   A   G   A   A   P
421/141                                                             451/151
GGG GAA GAA GAC CTG TGT GCA GCA TTT AAC GTC ATA TGT GAT AAT GTG GGG AAA GAT TGG
G   E   E   D   L   C   A   A   F   N   V   I   C   D   N   V   G   K   D   W
481/161                                                             511/171
AGA AGG CTG GCT CGT CAG CTC AAA GTC TCA GAC ACC AAG ATC GAC AGC ATC GAG GAC AGA
R   R   L   A   R   Q   L   K   V   S   D   T   K   I   D   S   I   E   D   R
541/181                                                             571/191
TAC CCC CGC AAC CTG ACA GAG CGT GTG CGG GAG TCA CTG AGA ATC TgG AAG AAC ACA GAG
Y   P   R   N   L   T   E   R   V   R   E   S   L   R   I   W   K   N   T   E
601/201                                                             631/211
AAG GAG AAC GCA ACA GTG GCC CAC CTG GTG GGG GCT CTC AGG TCC TGC CAG ATG AAC CTG
K   E   N   A   T   V   A   H   L   V   G   A   L   R   S   C   Q   M   N   L
661/221                                                             691/231
GTG GCT GAC CTG GTA CAA GAG GTT CAG CAG GCC CGT GAC CTC CAG AAC AGG AGT GGG GCC
V   A   D   L   V   Q   E   V   Q   Q   A   R   D   L   Q   N   R   S   G   A
721/241                                                             751/251
ATG TCC CCG ATG TCA TGG AAC TCA GAC GCA TCT ACC TCC GAA GCG TCC TGA TGG GCC GCT
M   S   P   M   S   W   N   S   D   A   S   T   S   E   A   S   *
781/261                                                             811/271
GCT TTG CGC TGG TGG ACC ACA GGC ATC TAC ACA GCC TGG ACT TTG GTT CTC TCC AGG AAG
841/281                                                             871/291
GTA GCC CAG CAC TGT GAA GAC CCA GCA GGA AGC CAG GCT GAG TGA GCC ACA GAC CAC CTG
901/301                                                             931/311
CTT CTG AAC TCA AGC TGC GTT TAT TAA TGC CTC TCC CGC ACC AGG CCG GGC TTG GGC CCT
961/321                                                             991/331
GCA CAG ATA TTT CCA TTT CTT CCT CAC TAT GAC ACT GAG CAA GAT CTT GTC TCC ACT AAA
1021/341                                                            1051/351
TGA GCT CCT GCG GGA GTA GTT GGA AAG TTG GAA CCG TGT CCA GCA CAG AAG GAA TCT GTG
1081/361                                                            1111/371
CAG ATG AGC AGT CAC ACT GTT ACT CCA CAG CGG AGG AGA CCA GCT CAG AGG CCC AGG AAT
1141/381                                                            1171/391
CGG AGC GAA GCA GAG AGG TGG AGA ACT GGG ATT TGA ACC CCC GCC ATC CTT CAC CAG AGC
1201/401                                                            1231/411
CCA TGC TCA ACC ACT GTG GCG TTC TGC TGC CCC TGC AGT TGG CAG AAA GGA TGT TTT TGT
1261/421                                                            1291/431
CCC ATT TCC TTG GAG GCC ACC GGG ACA GAC CTG GAC ACT AGG GTC AGG CGG GGT GCT GTG
1321/441                                                            1351/451
GTG GGG AGA GGC ATG GCT GGG GTG GGG GTG GGG AGA CCT GGT TGG CCG TGG TCC AGC TCT
1381/461                                                            1411/471
TGG CCC CTG TGT GAG TTG AGT CTC CTC TCT GAG ACT GCT AAG TAG GGG CAG TGA TGG TTG
1441/481                                                            1471/491
CCA GGA CGA ATT GAG ATA ATA TCT GTG AGG TGC TGA TGA GTG ATT GAC ACA CAG CAC TCT
1501/501                                                            1531/511
CTA AAT CTT CCT TGT GAG GAT TAT GGG TCC TGC AAT TCT ACA GTT TCT TAC TGT TTT GTA
1561/521                                                            1591/531
TCA AAA TCA CTA TCT TTC TGA TAA CAG AAT TGC CAA GGC AGC GGG ATC TCG TAT CTT TAA
1621/541                                                            1651/551
AAA GCA GTC CTC TTA TTC CTA AGG TAA TCC TAT TAA AAC ACA GCT TTA CAA CTT CCA TAT
1681/561
TAC AAA AAA AAA AAA AAA AAA
```

FIGURE 8

F2 clone

FIGURE 11

Yeda Research and Development Co. Ltd.

F9 clone

FIGURE 12

Yeda Research and Development Co. Ltd.

DD11 clone

....
AATTCGGCACGAGGTTGTGCTGTGGGAAGGGAGAAGGATTTGTAAACCCCGGAGCGAGGTTCTGCTTACCC
GAGGCCGCTGCTGTGCGGAGACCCCCGGGTGAAGCCACCGTCATCATGTCTGACCAGGAGGCAAAACCTTCc
AACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAAAGTCATTGGACAGGATAGCAGTGA
GATTCACTTCAAAGTGAAAATGACAACACATCTCAAGAAACTCAAAGAATCATACTGTCAAAGACAGGGTGT
TCCAATGAATTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGATAATCATACTCCAAAAGAACTGGG
AATGGAGAAGAAAGATTGTGATTtGAaGTTTTATCAGGAACAAACGGGGGGTCATTCAACAGcTT.......
....

FIGURE 13

Yeda Research and Development Co. Ltd.

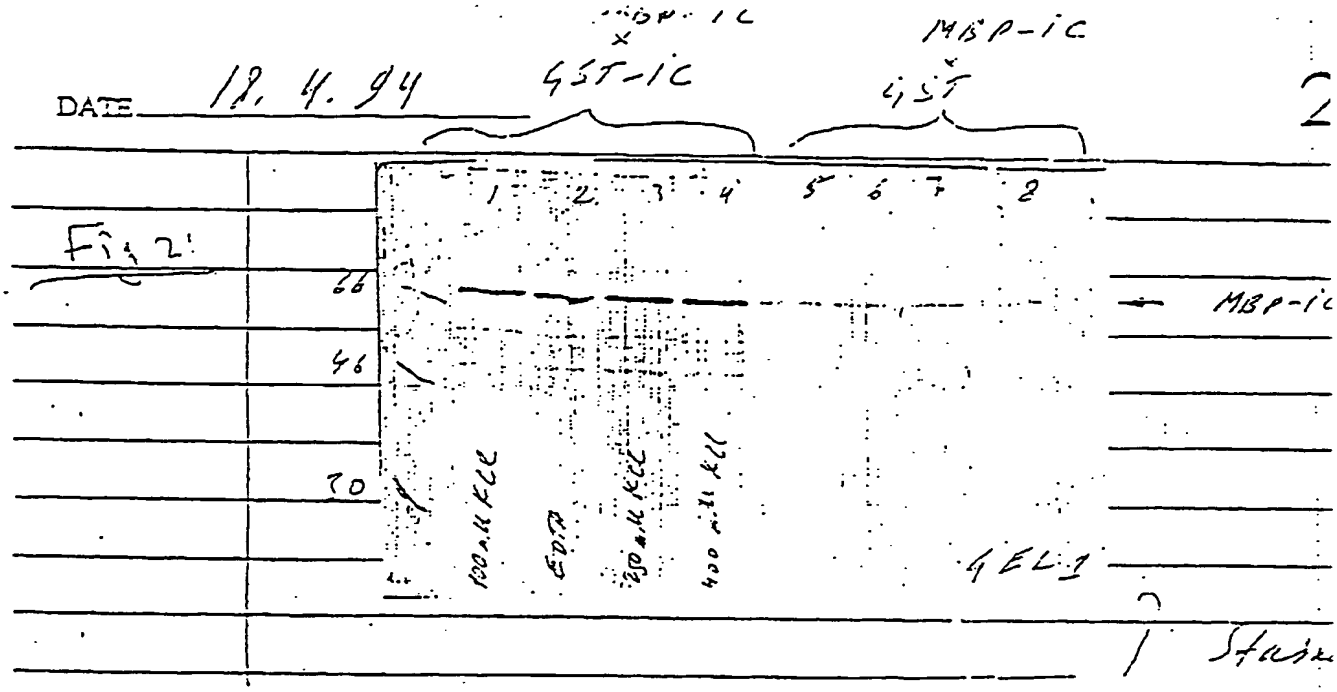

FIGURE 2

Yeda Research and Development Co. Ltd.

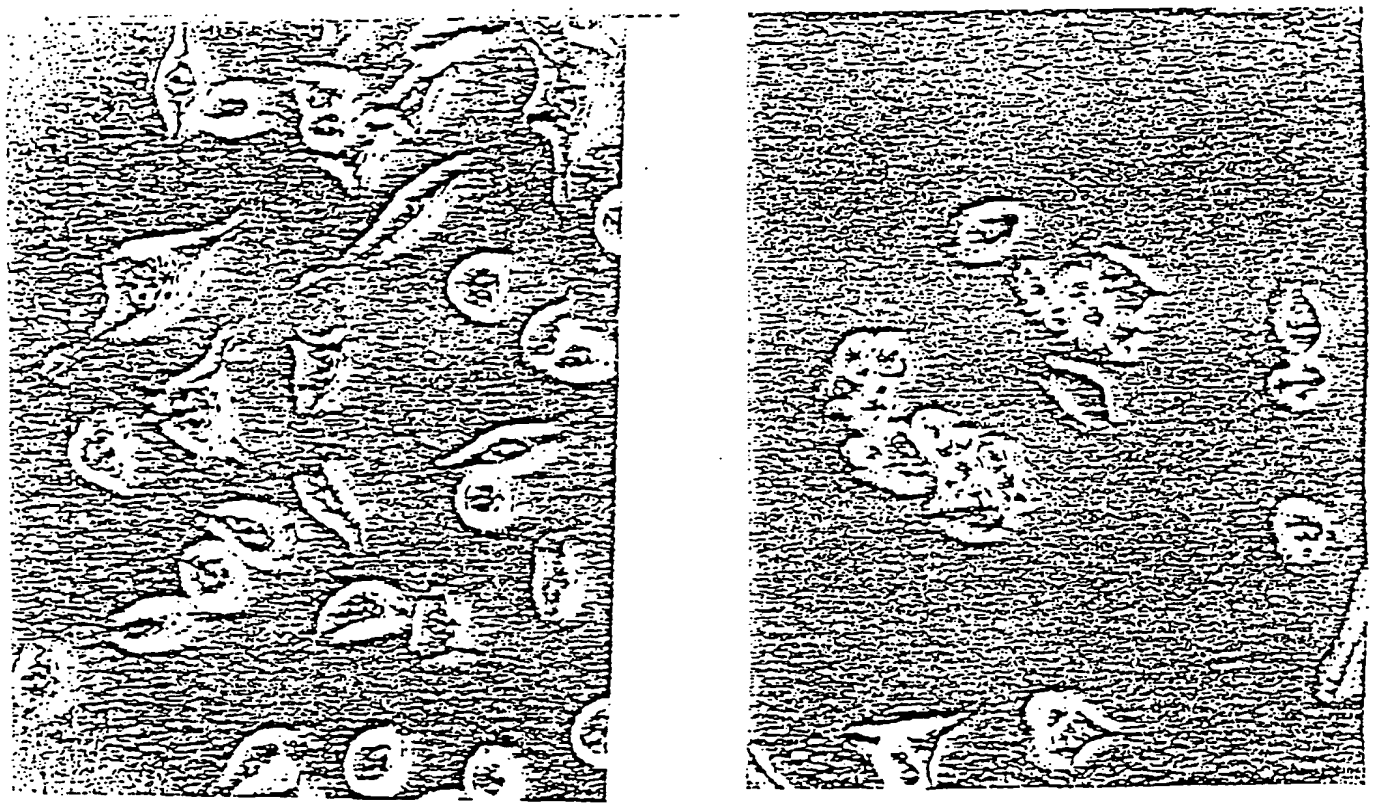

FIGURE 3

Yeda Research and Development Co. Ltd.

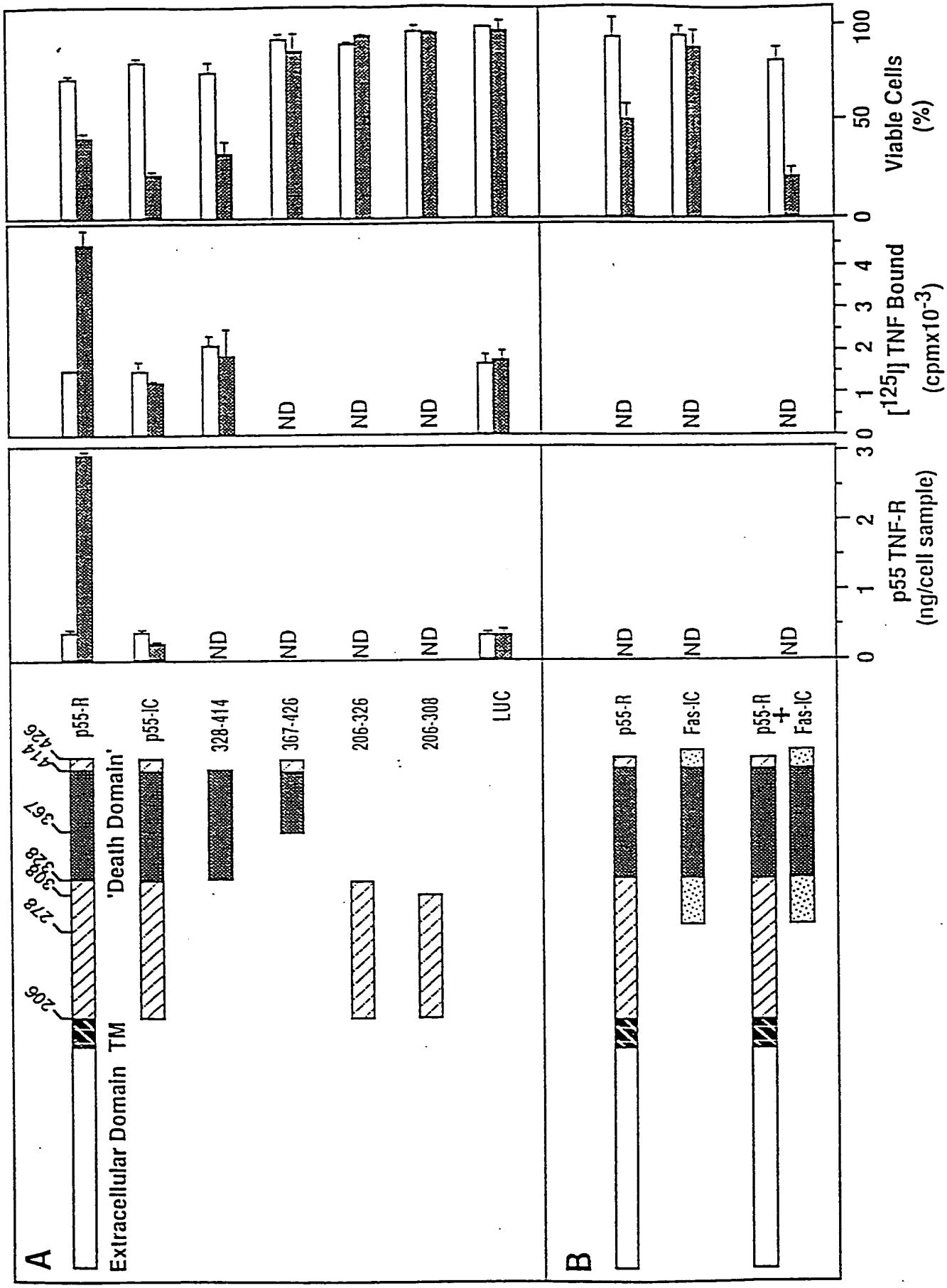

FIGURE 4

Yeda Research and Development Co. Ltd.

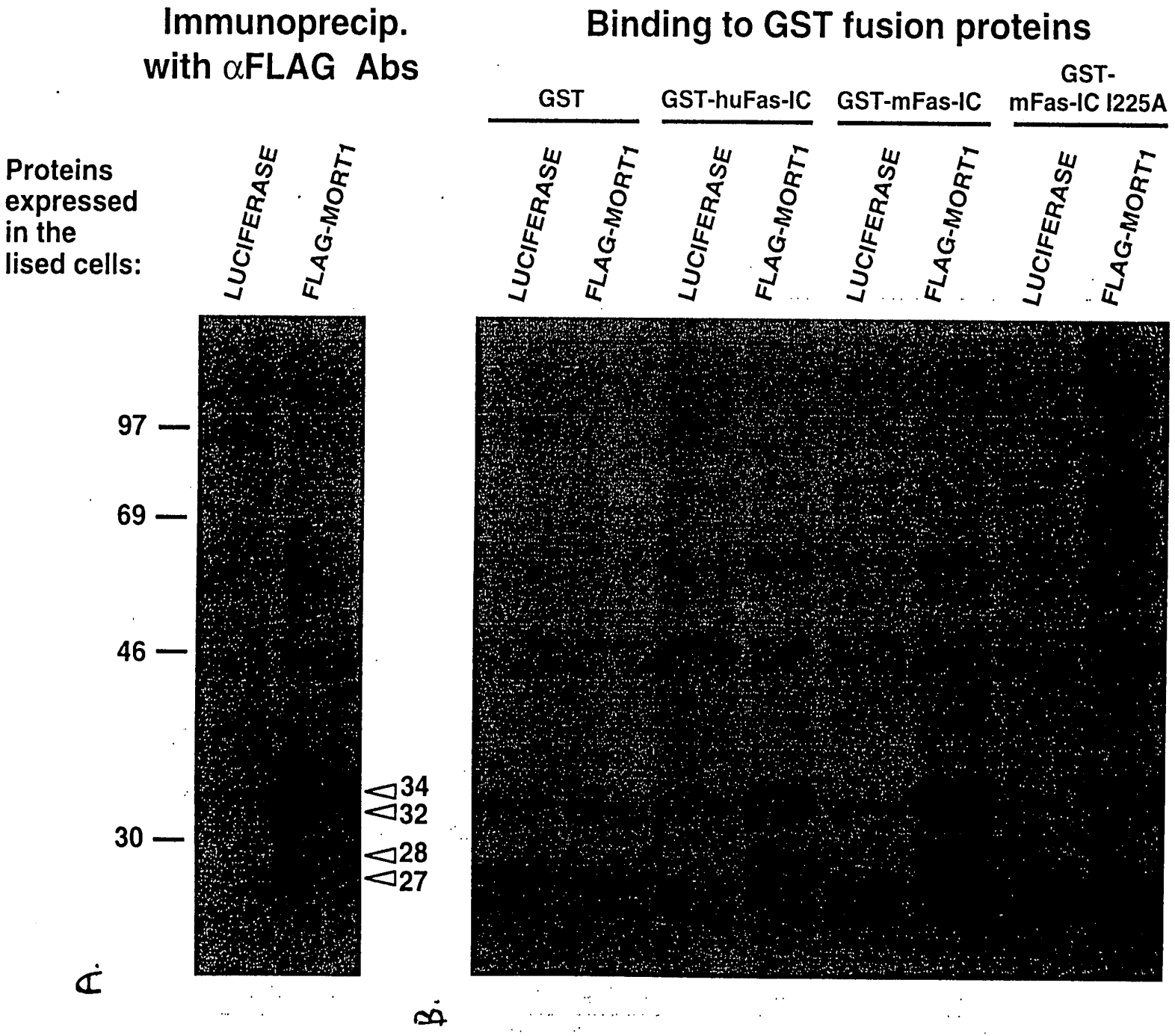
Immunoprecip.
with αFLAG Abs
Proteins
expressed
in the
lised cells:
LUCIFERASE
FLAG-MORT1
97
69
46
30
34
32
28
27
A.
Binding to GST fusion proteins
GST
GST-huFas-IC
GST-mFas-IC
GST-
mFas-IC I225A
LUCIFERASE
FLAG-MORT1
B.

FIGURE 5

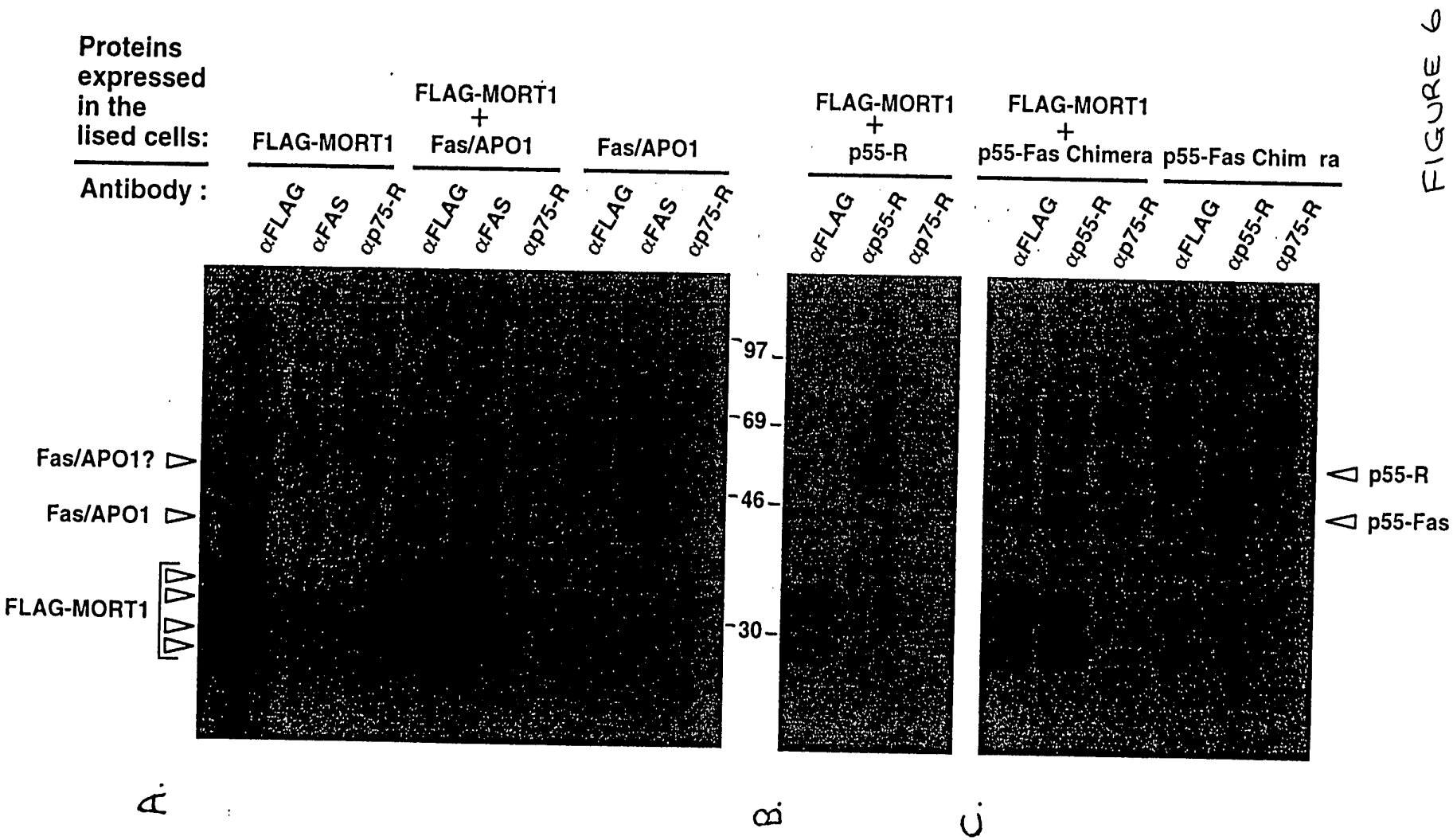
Proteins expressed in the lised cells:
Antibody :
FLAG-MORT1
FLAG-MORT1 + Fas/APO1
Fas/APO1
FLAG-MORT1 + p55-R
FLAG-MORT1 + p55-Fas Chimera
p55-Fas Chim ra
αFLAG
αFAS
αp75-R
αp55-R
Fas/APO1?
Fas/APO1
FLAG-MORT1
97
69
46
30
p55-R
p55-Fas
A
B
C

FIGURE 6

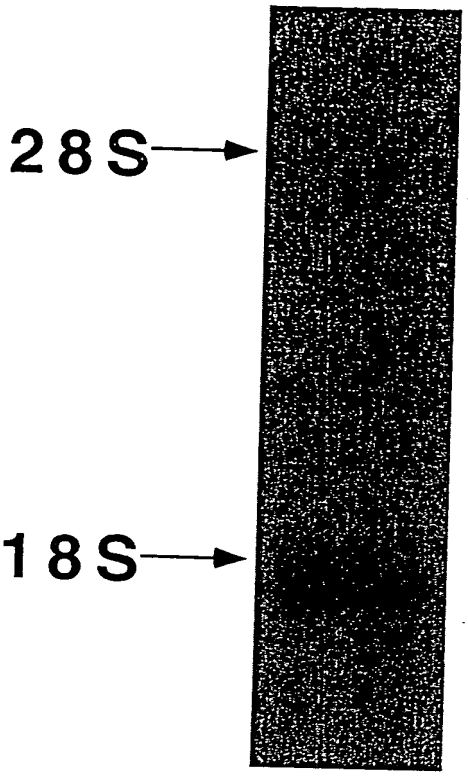

FIGURE 7

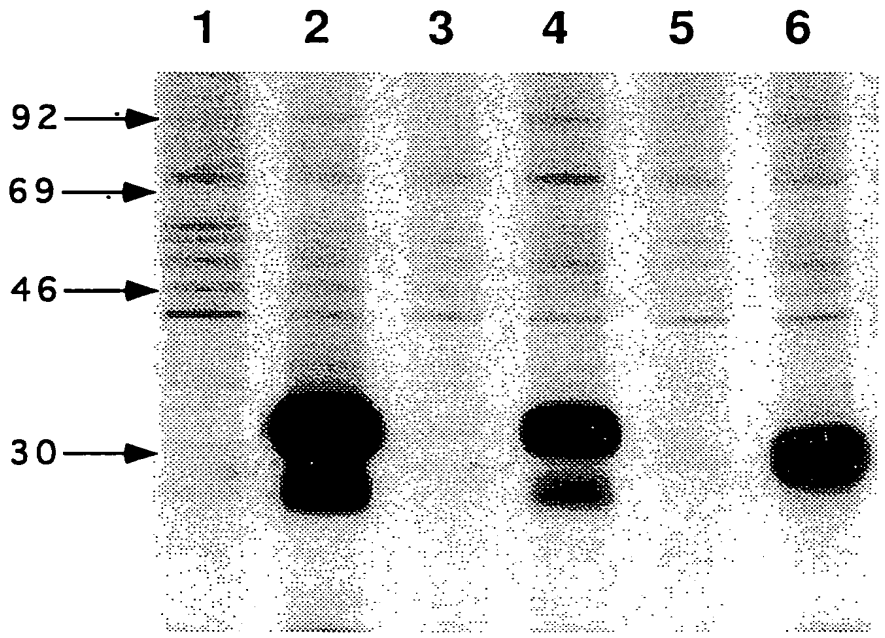

FIGURE 9

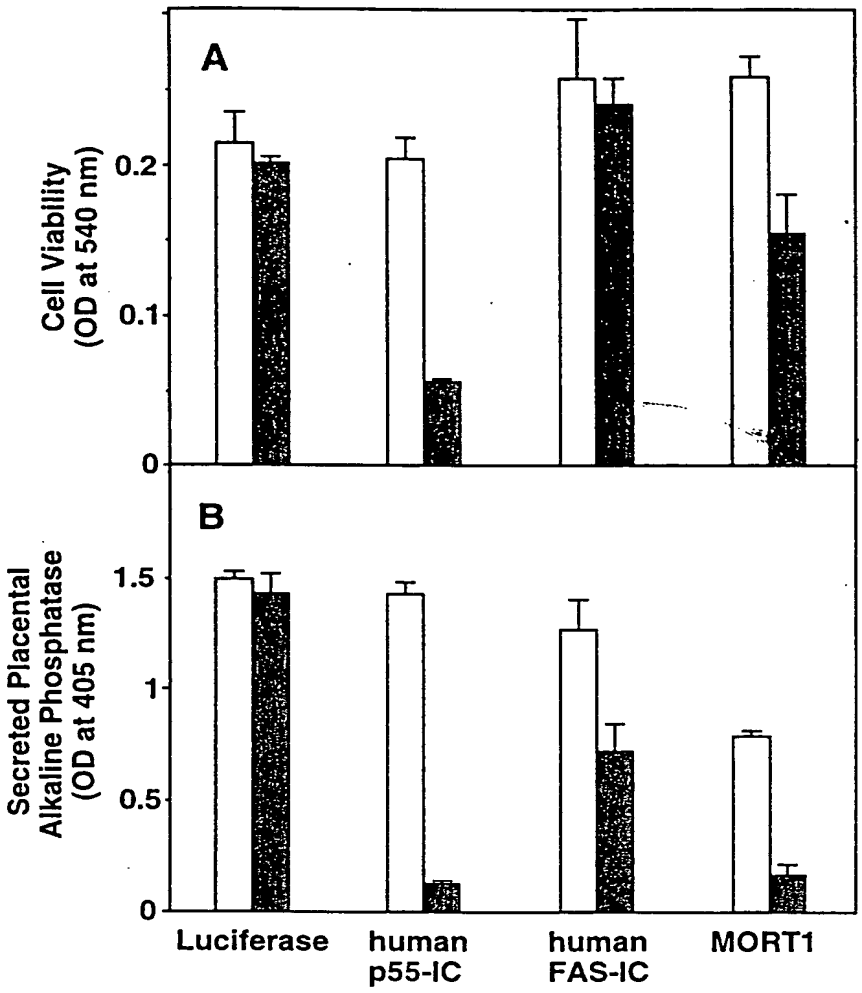
A
Cell Viability
(OD at 540 nm)
0.2
0.1
0
B
Secreted Placental
Alkaline Phosphatase
(OD at 405 nm)
1.5
1
0.5
0
Luciferase
human
p55-IC
human
FAS-IC
MORT1

FIGURE 10